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SOME PHYSIOLOGICAL RESPONSES OF THE SHEEP TO SHORT-TERM COLD EXPOSURE

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Some Physiological Responses of the Sheep to Short-Term Cold Exposure" submitted by Gerald John Mears, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

An experiment was conducted with shorn sheep to study the short-term effects of a low ambient temperature on jugular vein temperature, rectal temperature, packed cell volume, eosinophil count, and the plasma concentrations of sodium, potassium, calcium, magnesium, and glucose. The animals were placed singly in a chamber in which they were subjected during consecutive 3-day periods to air temperatures of 20.6 $\pm$ 1.0 C, 1.3 $\pm$ 1.5 C, and 19.2 $\pm$ 2.0 C.

Cold exposure of the sheep resulted in a decrease in the mean jugular vein temperature and the mean rectal temperature. The changes in the rectal temperature showed a thermal lag when compared to the jugular vein temperature.

During the cold period, an increase in packed cell volume occurred which did not, however, account for all of the changes observed in the various plasma parameters during cold exposure.

A large decrease in circulating eosinophils resulted when the sheep were exposed to cold, indicating adrenal cortical hyperactivity. In a preliminary trial, where unshorn sheep were moved between warm and cold rooms, the eosinopenia in the cold lasted about 12 hr, whereas in the main experiment with shorn sheep, the eosinopenia lasted for the full 54 hr of cold exposure.

An elevation of plasma sodium concentration accompanied the eosinopenia of cold exposure, indicating increased minerocorticoid activity.

During the first 12 hr of cold exposure, the relative plasma concentrations of potassium and magnesium were increased. Calcium showed a relative increase over the same period, but this may have been due to a

low initial calcium value in the cold period. Jugular vein temperature was depressed over the first 12 hr of the cold period indicating a temporary hypothermia which would lead to the observed increases in plasma potassium and magnesium.

Cold exposure of the sheep resulted in an elevation of plasma glucose concentration, especially during the first 12 hours. These changes in plasma glucose also suggested adrenal gland hyperactivity.

The movement of unshorn sheep from a warm to a cold room seemed to result in a temporary emotional stress rather than in cold stress. In the main experiment, however, the stressor agent appeared to be the cold. The sheep showed no signs of becoming acclimatized after 54 hr of exposure at 1.3 $\pm$ 1.5 C.

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INTRODUCTION

In Alberta, ambient temperature changes of 20 C or more may occur within the space of a few hours. Fluctuating winter temperatures, coupled with other environmental factors such as wind, can severely test the homeostatic mechanisms of animals exposed to these conditions.

Thermal regulation requires a balance between heat production and heat loss. The neurohormonal mechanisms which permit such a balance appear to be activated through the stimulation of peripheral, cutaneous, or of central, hypothalamic receptors, or are controlled by some combination of both receptor types.

The initial response to cold is the conservation of heat by such primarily physical mechanisms as peripheral vasoconstriction and piloerection. When the animal is no longer able to remain homeothermic by these mechanisms, increased chemical thermogenesis occurs. Adrenal gland hyperactivity plays a major role in increasing the rate of thermogenesis to balance the increased rate of heat loss. In severe cold stress, the circulatory and neurohormonal responses are accompanied by such activities as shivering and huddling.

Selye (1950) notes that a stressor agent is one which elicits systemic stress. Systemic stress is characterized by an extensive deviation from the normal resting condition of the body. In the broadest sense, any environmental factor affecting an organism so as to elicit physiological mechanisms which resist the change can be regarded as a stressor. Selye (1950) found cold to be an excellent means of producing nonspecific damage to tissues and used a lowered environmental temperature as a systemic stressor agent for many studies.

Cold exposure of a severity sufficient to cause systemic physiological

changes is often referred to as 'cold stress' in the literature. The term cold stress is used in this context in the present study.

Upon prolonged cold exposure, metabolic and physiological adaptations allow greater thermal conservation and provide for greater rates of non-shivering thermogenesis. With acclimatization, adrenal gland activity returns to normal.

If cold stress becomes extremely severe, hypothermia occurs. Hypothermia implies an abnormally low body temperature accompanied by a decrease in heart rate and respiration.

In order to allow an understanding of thermoregulation and acclimatization in animals, a more complete knowledge of the short-term physiological responses to cold is necessary. In this respect, changes in fluid and electrolyte balance have been shown to be important short-term responses to cold.

It has been demonstrated that, in small animals, marked fluid and electrolyte changes accompany adrenal responses to cold. In some of the studies reported in the literature, eosinophil counts have been used to demonstrate adrenal hyperactivity when the animal is cold stressed. Although the electrolyte balance changes reported in the literature show considerable inter- and intraspecies variability, much information concerning the effect of cold stress on hematocrit and on various plasma components such as sodium, potassium, calcium, magnesium, and glucose is now available for dogs, cats, rats, rabbits, hamsters, and pigeons.

For ruminant farm animals which are often exposed to the rapid changes in temperature previously mentioned, there is a paucity of information on such cold-dependent physiological responses as altered electrolyte balance and adrenal gland function.

In one study involving sheep, environmental comparisons were made

using a heated room and an unheated room (Hess, 1963). In this case there was a wide fluctuation in the cold room temperature. The results of a long-term study under these conditions indicated no significant changes in sodium, potassium, magnesium, or eosinophil count. In an experiment of shorter cold duration with a controlled cold room temperature (Bailey, 1964), cold exposure led to significant changes in plasma sodium and packed cell volume but not in plasma potassium, calcium, or magnesium.

Cold stress in conjunction with wind and rain (Reid, 1962) or fasting (Weeth, Torell, and Cassard, 1959) caused an increased adrenal function in the sheep.

The experiments reported herein were undertaken at the University of Alberta to determine the extent to which short-term cold exposure causes stress in the sheep, through the study of eosinophil counts and of changes in a number of plasma parameters which occur during the early stages of cold exposure.

REVIEW OF LITERATURE

Physiological Reactions to Cold

I. Thermoregulation

A. Effects on body temperature

In general, the extent to which rectal temperature falls in cold-exposed animals depends on the animal's ability to withstand cold as well as on the intensity of cold (Platner and Hosko, 1953; Dukes, 1955). The sheep is well insulated by means of its fleece, and appears to be quite cold resistant.

Hess (1963) reported a significant ($P < 0.01$) decrease from 38.9 to 38.8 C in the average rectal temperature of sheep exposed to long-term cold. These results were not duplicated by Bailey (1964) when sheep during short-term exposure to cold maintained the same rectal temperature as their controls. However, subcutaneous temperatures decreased slightly. In both sites, temperature decreased at first but rose again towards the end of the week of cold exposure.

Rectal temperature, because of its thermal inertia, is not a reliable index of deep body temperature (Benzinger, 1961; Bligh, 1957; Ross, 1956; Veeraraghavan, 1963). Of 14 subcutaneous tissue and skin sites examined, jugular vein blood temperature was the only one which closely corresponded to the temperature of the blood bathing the thermoregulatory nuclei of the hypothalamus via the internal carotid artery (Veeraraghavan, 1963). Temporary hypothermia caused by the addition of ice water to the rumen resulted in an almost immediate drop in the intravascular temperature which took about 160 min to return to pretreatment temperatures. On the other hand, rectal temperatures reacted much slower initially and did not return to pretreatment temperature for 6 to 8 hours.

B. Physical regulation

At temperatures below the thermal neutral point, about 75% of the heat lost from the body is by radiation, conduction, and convection and about 25% is lost by evaporation from the skin and lungs (Dukes, 1955). Smaller amounts of heat are lost in the feces and urine. Heat loss is increased by vasomotor mechanisms such as sweating and increased pulmonary ventilation. Heat loss can be augmented by redistribution of tissue water (Evans, 1956). Heat conservation is brought about by vasoconstriction and piloerection (Dukes, 1955) and by countercurrent heat exchange involving the rete mirabile (Scholander, 1957).

C. Chemical regulation

When physical mechanisms of heat conservation are no longer able to stem the additional heat loss in the cold, additional heat is produced by oxidative processes (Evans, 1956; Carlson, 1963). This includes shivering thermogenesis (Dukes, 1955) and nonshivering thermogenesis (Hart, 1958; Carlson, 1960; Chaffee et al., 1963; Davis, 1963; Jansky and Hart, 1963).

Glucocorticoid production is rapidly increased by cold stress (Hart, 1958; Reid, 1962). Severe cold stress increases norepinephrine and epinephrine secretion by the adrenal medulla and sympathetic nerve endings (Carlson, 1960; Hannon, Evonuk, and Larson, 1963; Hart, 1958; Reid, 1962; Sellers and Schönbaum, 1963). The glucocorticoids stimulate gluconeogenesis and the epinephrine stimulates glycogenolysis (Hannon, 1960; Reid, 1962; White et al., 1959). Both result in elevated blood glucose levels.

As the animal begins to adapt to its cold environment, thyroid hyperfunction takes over from adrenal hyperfunction (Boulouard, 1963; Hart, 1958; Héroux, 1960; Knigge, 1963; Nicholls and Rossiter, 1956). One

of the characteristic results of thyroid gland hyperfunction is hyperglycemia (White et al., 1959).

Rats cooled at the rate of about 5 C per hour showed rapid utilization of liver glycogen and, when ample stores of carbohydrates were present, showed a rapid and prolonged increase in blood glucose (Fuhrman and Crismon, 1947). This conversion of glycogen to glucose is a result of nervous and endocrine activity (Fuhrman and Fuhrman, 1963). When glucose was administered to hypothermic humans and dogs, a marked and sustained hyperglycemia resulted indicating the very slow metabolism of glucose during hypothermia (Wynn, 1954). In a woman given a total of 74 g of glucose in 9 hr, the plasma glucose rose 962 mg/100 milliliters. This would indicate that the glucose was diluted to about 7.7 liters which was 16% of the woman's body weight. It would appear that no exogenous glucose was metabolized as the extracellular fluid weight in a woman is about 16% of the body weight. The same glucose load given at normothermic temperatures would have hardly altered the blood sugar level. Henneman, Bunker, and Brewster (1958) reported that cooling the human body to 28 to 30 C raised blood sugar levels and that when 5 to 15 g of dextrose were infused, a marked hyperglycemia occurred. They suggested that there is an inhibition of the tissue uptake of glucose and of glycolysis. In order to investigate this effect, Fuhrman and Fuhrman (1963) injected C^{14}-labeled glucose into fasted hypothermic rats and found that the specific activity of the glucose remained constant in the blood for 2 1/2 hours. Conditions for glucose uptake should have been optimal as these rats were fasted. Also, the glycogen levels in the livers of cooled rats were the same with and without glucose loading. It appeared that failure to convert glucose to glycogen was the cause of hyperglycemia in hypothermia.

The penetration of glucose into tissue cells at low temperature apparently equals or exceeds the rate of phosphorylation, according to more recent studies done by Fuhrman and Fuhrman (1964). Thus, the abnormal metabolism of glucose in hypothermia cannot be attributed to an inhibition of the tissue uptake of glucose. Soluble glycogen is more labile than residual glycogen during hypothermia (Platner, Shields, and Purdy, 1964). The incorporation of glucose-C^{14} into tissue glycogen of hypothermic rats was increased in soluble and residual fractions of the heart but not in liver or muscle.

Glucagon injected into normothermic rats produced hyperglycemia but not when administered to hypothermic rats (Crawford et al., 1965).

Reid (1962) found increased blood glucose levels in ewes when exposed to cold, wind, and, in some cases, rain. Pregnant ewes showed less response than nongravid ewes. Fasting of 24 hr or more before going on trial caused larger sustained increases in blood glucose concentrations as these sheep were already in a state of gluconeogenesis. A shorter fasting period resulted in a temporary increase and then a decline after 2 to 4 hr of stress. Some sheep then stabilized at this point while in others blood glucose rose rapidly several hours later. This was attributed to increased adrenaline secretion causing glycogenolysis in the early stages. As glycogen stores became depleted, a reduction of the elevated blood glucose occurred. Then gluconeogenesis was believed to occur in some animals with a second elevation of blood glucose being the result.

In the same experiment it was found that elevated glucose levels were not maintained with only wind and cold stress. It was suggested that the major part of this temporary increase in the sheep was due to adrenaline secretion via glycogenolysis.

Some of the reasons for increased blood glucose levels without a turnover of liver glycogen might be obtained from studies on cold-adapted animals. Hannon (1960) noted changes in the intermediary metabolism in cold-acclimatized rats. These animals showed increases in the activity of both glucokinase and glucose-6-phosphatase. Phosphorylase activity was decreased and became only 25% as active as phosphoglucomutase. It appeared that the rate of glycogenolysis was limited by the low level of phosphatase activity. However, epinephrine production was shown to be high in rats chronically exposed to cold (Hart, 1958) and epinephrine has been shown to work at the phosphorylase level in glycogenolysis (Hannon, 1960; White et al., 1959).

D. Thermostatic control

Ranson (1937) demonstrated the presence of heat-regulating centers in the anterior hypothalamus of the monkey where lesions led to a loss of the ability to withstand high temperatures. Magoun et al. (1938) heated the anterior hypothalamus of the cat and caused vasodilation, panting, and sweating. Local temperature changes within the hypothalamus affect heat production in rats and cats as well as muscular activity and blood flow in dogs (Carlson, 1962). Repin (1963) obtained abrupt abolition of muscular activity by electrical stimulation of the preoptic area of chilled rabbits. Chowers et al. (1964) showed that acute lowering of environmental and preoptic temperature evoked a rise in plasma cortisol levels. A number of other workers have implied a thermostatic control by the hypothalamus in monogastric animals (Adams, 1963; Fusco, Hardy, and Hammel, 1961; Hammel et al., 1963; Han and Brobeck, 1961; Petajan, Morrison, and Akert, 1962).

Lee (1950) suggests that the heat-regulating centers of the sheep hypothalamus are probably sensitive to very small changes in blood temper-

ature.

Andersson, Grant, and Larsson (1956) and Andersson et al. (1963, 1964a, 1964b) demonstrated a discrete 'heat-loss center' in the goat. Electrical stimulation and local cooling of the preoptic region of the anterior hypothalamus caused core hyperthermia in goats. A marked increase in the release of protein-bound iodine from the thyroid and increased urinary catecholamines resulted from the cold treatment. The use of a ganglionic blocking agent prevented core hyperthermia, increased the excretion of urinary catecholamines, and reduced shivering. However, heating the 'heat-loss center' in a cold room caused a rapid drop in core temperature. These changes demonstrate that the hypothalamus is important in the control of body temperature in the goat.

On the other hand, Blaxter (1962) feels that ruminants are distinctly different from humans in that the peripheral receptors are very important in the control of body temperature in the ruminant animal in warm environments. Hammel et al. (1963) points out that a combination of both peripheral and central temperature sensitive receptors causes responses in the rhesus monkey and the dog. Carlson (1963) and Fusco et al. (1961) support the latter view. This may very well be the case in ruminants, also.

II. Environmental cold stress and the adrenal gland

Selye (1950) lists cold as a stress which causes hyperfunction of the adrenal gland. In the first phase of cold acclimation, the rat is unable to compensate for increased energy expenditure by a sufficient food intake and is obliged to use its energy reserves aided by a concomitant adrenocortical hyperactivity (Boulouard, 1963). In rats suddenly exposed to cold there is a rapid increase in the excretion of urinary 17-oxosteroids with the levels falling to subnormal values within 36 to 48 hr (Munday and

Blane, 1960).

Schönbaum (1960) reported that exposure of rats to acute cold caused a transient rise of corticosteroid formation during the first 30 min of cold exposure. Boulouard and Buzalkov (1963) and Héroux (1960) reported increased secretion of adrenocortical hormones during early cold exposure. Work by Héroux and Hart (1954) and Yamashita and Araki (1962) supports the theory of adrenal hyperactivity during the early stages of cold exposure.

Cholesterol, which has been shown to be the precursor of the adrenal cortical hormones, is normally found in large amounts in the adrenal cortex. Cold shock rapidly decreases these levels in the cortex (Dukes, 1955). Ascorbic acid is normally found stored in the adrenal cortex and disappears rapidly during acute cold, apparently entering into the synthesis of adrenalcortical hormones (Booker, 1960; Hoijer, 1960).

Hume and Egdahl (1959) found that hypothermia does not at any time act as a stimulant to adrenal cortical secretion, and Evans (1956) reports hypothermia causes a reduction of adrenal cortical function.

A good indicator of adrenal gland activity was found to be the number of circulating eosinophils (Miller, 1955). The adrenal hyperactivity during stress greatly reduced the number of circulating eosinophils (Speirs and Meyer, 1949). Weeth et al. (1959) found pronounced eosinopenia in sheep exposed to cold, indicating adrenal hyperactivity. Selye (1950) noticed pronounced leukopenia during the 'alarm reaction' of severe cold stress. Yamashita and Araki (1962) immersed dogs in ice water for short periods. After removal of the dogs from the ice water, peripheral blood showed a marked decrease in the number of circulating eosinophils with the greatest decrease occurring 2 to 4 hr after removal from the bath. This

occurred in both the intact and adrenal demedullated dog, whereas no changes in circulating eosinophils occurred in adrenalectomized dogs. Their conclusion was that the eosinopenic responses of the intact dog in cold is mainly due to increased adrenal cortical activity. Hess (1963) reported a nonsignificant difference in eosinophil counts in sheep during long-term exposure to cold. The mean eosinophil count for the sheep kept in a warm room at 12.8 to 18.3 C was 313/mm^3 as against 350/mm^3 for the sheep kept at -18.9 to 13.3 C in the unheated room. This trend towards eosinophilia which took place is a reversal of the usual and suggests that the sheep were under no stress.

III. Electrolyte responses to cold

It is believed that the observed plasma electrolyte responses during cold exposure are the result of early hyperactivity of the adrenal gland with the release of minerocorticoid hormones (Munday and Blane, 1961). Significant alterations in electrolyte metabolism indicate that posterior pituitary and electrolyte-regulating hormones are mobilized as well as adrenocorticotropin and thyrotropin in the complex response evoked by cold exposure (Knigge, 1960).

Evidence for increased minerocorticoid in early cold stress is further substantiated by the fact that adrenalectomy, which prevents the normal eosinopenia due to mild stress or epinephrine injections in mice (Speirs and Meyer, 1949), prevented the fluctuations of plasma electrolytes in cold-stressed rats (Munday and Blane, 1960). Furthermore, adrenalectomy in dogs (Swingle and Swingle, 1965) and in rats (Leonard, 1963) and the use of spironolactone, an aldosterone antagonist (Rapp, 1964; Rovner et al., 1963), prevented the normal distribution and movements of sodium and water in normothermic animals.

The major minerocorticoid released by the adrenal cortex is aldosterone which is widely known as the sodium-retaining hormone (Denton, Goding, and Wright, 1960; Laragh and Stoerk, 1957). Aldosterone secretion may be under the control of the hypothalamus (Sims and Solomon, 1963) or under the control of the renin-angiotensin system in the kidney (Binnion et al., 1965; Denton, 1964; Sims and Solomon, 1963; Slater et al., 1963).

A. Sodium

Plasma from rats acclimatized at 5 C contained significantly higher sodium levels than plasma from rats maintained at a control temperature of 26 C (Hannon, Larson, and Young, 1958). The first day of cold exposure (2 C) resulted in no change in plasma sodium in rats (Baker and Sellers, 1957). Continued exposure resulted in retention of sodium and after 45 days in the cold a sustained elevation of plasma sodium had occurred. The intracellular sodium of muscle was decreased by sustained exposure of the rat to 2 C (Baker, 1960a). Munday et al. (1958) noted a small sustained rise in sodium levels in hypothermic rats, rabbits, and humans. Munday and Blane (1960) found transient high plasma sodium levels in rats exposed to severe cold at 0 C for 48 hours. The sodium concentration of the urine was simultaneously reduced. These changes did not occur in adrenalectomized rats similarly exposed.

Pigeons, which have a greater resistance to cold than the rat, showed similarly high plasma sodium values when exposed to 0 C for 48 hr but returned to normal more rapidly (Munday and Blane, 1961).

Rats subjected to a long-term cold exposure of 4 to 20 wk at 0 C were found to have an increased plasma sodium concentration, but this was in conjunction with an increase of plasma potassium which was opposite to the effect found during short-term cold exposure (Munday and Blane, 1961).

Beaton (1961) cooled fasted rats in ice until rectal temperatures of 15 C were achieved but found no alteration in red cell and plasma sodium. Spurr and Barlow (1959) found an apparent shift of sodium from plasma into erythrocytes during hypothermia in dogs.

Steadman, Ariel, and Warren (1943) exposed rabbits to cold for 2 to 5 hr and found serum sodium levels fluctuated from -20 to 19% when the plasma samples taken immediately after hypothermia were compared with the samples taken before the cold treatment. No explanation for the wide fluctuations was offered.

Immersing sheep in ice water and lowering body temperatures to 28 to 30 C resulted in higher sodium values in skeletal muscles (Hercus and Bowman, 1959). This either resulted from the accompanying anoxia caused by hemodynamic changes or was due to a direct depression of cell metabolism, reducing the rate of energy turnover below that necessary to maintain the sodium pump.

Hess (1963) measured plasma sodium in eight yearling wethers three times each week for 43 days. The control temperatures were 12.8 to 18.3 C while cold room ambient temperatures varied from -18.9 to 13.3 C with an average of -5 C. The wool length was approximately one inch. The plasma sodium concentration in the cold-treated animals did not differ significantly from the mean plasma sodium value of 152.4 meq/l found in the control animals. There was a significant increase ($P < 0.01$) in the urinary sodium concentration in the cold-treated animals from 2.54 to 4.30 meq/liter. Part of the increase in urine sodium concentration could be accounted for by a 29% reduction in urine volume (significant, $P < 0.01$). However, a greater absolute amount of sodium was excreted in the urine during cold exposure. There was no significant correlation in this study

between degree-hours* and plasma sodium, but there was a significant correlation ($P < 0.05$) between degree-hours and urine sodium.

Bailey (1964) used four 3-year-old wethers, sheared 6 weeks before the experiment, to study short-term fluctuations in temperature and the effects of different water consumption levels. The sheep had free access to water in one period or received 1000, 2000, or 3000 g of water per day through a fistula in each of the other periods. The experimental treatments consisted of 1 week at 20 C, 1 week at -11 C, and a 3rd week at 20 C. Samples were taken three times weekly. When the water intake was voluntary, the mean plasma sodium value of 160 meq/1 found in the cold was significantly ($P < 0.05$) higher than the mean values of 149 and 152 meq/1 for the first and second warm-temperature periods, respectively. When the water intake was limited to 1000 g, the same significant differences occurred. When the water intake was 2000 and 3000 g, no significant differences in plasma sodium concentration resulted. This suggests that the changes in the first two instances were due to changes in the concentration of the blood which were indicated by the packed cell volume.

B. Potassium

When sodium is retained due to the effect of aldosterone on the kidney, potassium is excreted in an exchange for sodium and hence a lower plasma potassium concentration may occur (Horton and Biglieri, 1962; Munday and Blane, 1960; Sims and Solomon, 1963). However, plasma potassium levels may increase rather than decrease during cold exposure. Langdon and Kingsley (1964) suggest that the acidosis accompanying hypothermia and the depression in function of cell membrane at low temperatures are at

*Degree-hours per day = the daily total differences between each recorded hourly temperature and 65 F; the colder the day, the greater the number of degree-hours.

least partly responsible for the increase in serum potassium. Entry of potassium into the skeletal muscle and into the spinal cord from the blood stream is retarded by cold exposure of the rabbit (Pogosova, 1959).

Hannon et al. (1958) found no significant differences in plasma potassium levels when rats were kept at 26 C or acclimatized at 5 C. Beaton (1961) observed no changes in the plasma potassium levels of fasted rats cooled in ice to a rectal temperature of 15 C, but there was a significant decrease in erythrocyte potassium.

Swan et al. (1953) found consistent decreases in serum potassium in hyperventilated dogs and humans during severe hypothermia and suggested that a shift of potassium to the intracellular phase existed, since they could not account for the loss in the urine. Spurr and Barlow (1959) noted a decrease in plasma potassium in hypothermic and in normothermic, hyperventilated dogs, whereas a potassium elevation in erythrocytes only occurred in the normothermic dog after ventilation. Kanter et al. (1963) used ice packing to lower the rectal temperatures of dogs and found a lowered potassium concentration in the blood despite a fall in arterial pH which will not occur in normothermic animals. This led to the suggestion of a potassium-for-sodium exchange in the renal tubule, with potassium being excreted instead of hydrogen.

Munday et al. (1958) noted a marked fall in plasma potassium in hypothermic rats, humans, and rabbits. Munday and Blane (1960, 1961) noted a decrease in plasma potassium for reptiles and rats but no similar decrease for pigeons when subjected to cold at 0 C for 48 hours. This is in agreement with the observation that the pigeon has a greater ability to withstand cold. The level of potassium in the urine was increased for the first 12 hr of cold exposure in the rat.

During long-term cold stress in the rat (4 to 12 weeks at 0 C), the plasma potassium levels were high (Munday and Blane, 1961).

During profound hypothermia in man, plasma potassium levels rose but there was not a proportionate decrease in urinary loss of potassium (Langdon and Kingsley, 1964).

Elliott and Crismon (1947) reported small but statistically significant increases in plasma potassium in rats having lowered body temperatures. Baker (1960b) noted an increase in rat plasma potassium for the first few days of cold exposure but found that after prolonged cold exposure this returned to normal.

In hypothermic sheep, serum potassium was lowered and an examination of muscle specimens revealed that intracellular potassium was diluted by the entrance of sodium and water (Hercus and Bowman, 1959).

In the experiment described earlier, Hess (1963) found that the mean plasma potassium concentration of 5.48 meq/l for sheep in the cold room was not significantly different from the mean plasma potassium concentration of 5.33 meq/l for sheep in the warm room. The urine from the cold-room sheep contained a significantly ($P < 0.01$) greater concentration of potassium than the urine from the warm-room sheep. Although it might have been expected that the increase in urine potassium concentration could have been due to the significant ($P < 0.01$) 29% reduction in urine volume, it was found that a greater absolute amount of potassium was excreted in the urine during cold exposure. There was no significant correlation between either plasma or urine potassium and degree-hours.

Bailey (1964), in the experiment previously described, found a nonsignificant increase in plasma potassium of sheep exposed to short-term cold and receiving either a voluntary water intake or 1000 g of water

intraruminally per day. When 2000 or 3000 g of water per day were administered intraruminally, the levels of plasma potassium remained the same for the cold-treated sheep. The small increases in plasma potassium were possibly due to hemoconcentration accompanying the cold exposure.

C. Calcium

There is evidence that the renal excretion of calcium in man is under the influence of the adrenal steroids and that calcium excretion decreases parallel to the sodium:potassium ratio in plasma (Lamberg and Torsti, 1964). The calcium concentration in the blood is very stable and depends primarily on the relationship between bone calcium and the parathyroid glands (Kunin, 1963).

Elliott and Crismon (1947) found a small but statistically significant increase in plasma calcium in rats with body temperatures cooled to 25 C. The calcium:potassium ratio of 1.46 in normothermic rats was reduced to 1.08 in the hypothermic rats. Injection of calcium chloride was shown to help protect the rats against hypothermia by lowering the lethal cold temperature.

However, Beaton (1961) found no changes in the level of serum calcium when fasted rats were cooled with ice packs until their rectal temperatures were lowered to 15 C. Hannon et al. (1958) using cold-acclimatized rats and Nowell and White (1963) using cold-stressed rats found no significant changes in plasma and serum calcium concentrations.

Bailey (1964), in the previously described experiment, reported that the plasma calcium concentration in sheep receiving a voluntary water intake rose nonsignificantly from 5.4 to 5.5 meq/l when the sheep were placed in the cold room. The results were similar when 1000 g of water per day were administered intraruminally but were reversed when the sheep

were given 2000 or 3000 g of water per day by the same route. There may have actually been a decrease in plasma calcium during cold exposure because of the decrease in plasma volume indicated by the packed cell volume.

D. Magnesium

Aldosterone has often been shown to influence magnesium metabolism, with increased circulating aldosterone levels causing an increased excretion of magnesium and a lower plasma magnesium concentration (Care and Ross, 1963; Cope and Pearson, 1963; Hanna and MacIntyre, 1960; Horton and Biglieri, 1962; Scott and Dobson, 1965). Deoxycorticosterone (Care and Ross, 1963) and cortisone (Aikawa, Harms, and Reardon, 1960) similarly affect magnesium balance.

Usually cold stress has the opposite effect and elevates plasma magnesium concentration. The cause of serum magnesium elevation in cold is not adrenal hyperactivity (Neubeiser, Platner, and Shields, 1961) but may be due to the loss of intracellular magnesium from hypothermic skin tissue (Moussa and Boba, 1960). The increased excretion of magnesium in the cold may spare the body of the pharmacological peripheral actions of magnesium on vasodilation and paralysis of the neuromuscular junction (Heagy and Burton, 1947). The increase in plasma magnesium may also be a result of the decrease in metabolic energy necessary to maintain the concentration gradient (Hannon et al., 1958).

Steadman et al. (1943) reported that short-term cold stress caused a mean serum magnesium increase of 24% in rats and that the levels were not related to the duration of the cold exposure (2 to 5 hr) nor to the degree of cold (13 to 22 C). It was suggested that the elevation of serum magnesium may have been due to an impairment of kidney function preventing magnesium

excretion, or that the magnesium may have been prevented from returning to its storage sites by the cold state.

Hannon et al. (1958) found a significantly higher plasma magnesium level in rats acclimatized at 5 C than in rats maintained at a control temperature of 26 C. Baker (1960b) found that plasma magnesium concentrations were not elevated in rats during the initial stages of cold exposure but that, after prolonged cold exposure, magnesium elevation did occur.

Nowell and White (1963) found an inverse relationship between daily photoperiod and changes in serum magnesium in rats exposed to cold. Cold exposure during the winter months resulted in a 24% increase in serum magensium, while cold exposure during the summer months resulted in a nonsignificant fall of serum magensium. There was no difference between cold-acclimatized and control rats with respect to the rise in plasma magnesium during cold stress (Nowell and White, 1964).

The known pharmacological actions of magnesium are peripheral vasodilation and paralysis of the neuromuscular junction (Heagy and Burton, 1947). The injection of 1 M magnesium chloride into dogs that were regulating against heat and intermittently panting caused an increase in the frequency and intensity of panting and a concomitant depression of body temperature. In dogs that were neither panting nor shivering, magnesium caused vasodilation and temperature depression, while magnesium had little effect on dogs that were regulating against cold by shivering. However, when high levels of magnesium were administered, paralysis, accompanied by hypothermia, resulted.

Moussa and Boba (1960) reported that dogs will exhibit a linear increase in plasma magnesium relative to the fall in core temperature.

Administration of magnesium sulphate during hypothermia caused increases in plasma magnesium levels and also raised the lethal temperature. It therefore appears unlikely that the rise in plasma magnesium is the primary factor influencing core temperature. The changing plasma magnesium seems to be primarily a reflection of decreased magnesium storage in hypothermic tissue.

Platner and Hosko (1953) also found that the rate of increase of serum magnesium appears to depend on the rate of fall of the body temperature. When the increase of serum magnesium concentration was expressed per degree centigrade of fall in the rectal temperature, turtles, puppies, hamsters, rats, cats, and mature dogs ranked in this decreasing order between 0.175 and 0.037 mg of magnesium per 100 ml of plasma per degree centigrade rectal temperature fall. This was in agreement with each of these animal's ability to resist a fall in body temperature when exposed to cold. The increase in serum magnesium may occur because a stimulus releases calcium into the cell interior probably replacing the divalent magnesium ion which is then released from the cell. This may be a passive movement resulting from a decreased rate of cellular metabolism in the hypothermic areas of the body.

In sheep exposed to long-term cold (Hess, 1963), the mean plasma magnesium level was the same as for the control animals (0.9 meq/1) and the urine magnesium concentrations were the same. Consequently, there was no significant correlation between degree-hours and urine or plasma magnesium. However, a greater absolute amount of magnesium was excreted in the urine during warm exposure.

Bailey (1964) found that short-term cold stress in sheep resulted in a nonsignificant increase in the mean plasma magnesium concentration from 2.6 to 2.9 meq/liter. These plasma magnesium values were about the same as

the values reported for most other species, whereas the magnesium concentrations reported by Hess (1963) were abnormally low. These two workers used essentially the same method of chemical analysis for magnesium.

IV. Changes in body-fluid compartments

Cold exposure is accompanied by changes in the body-fluid compartments (Bass and Henschel, 1956; Evans, 1956). Some authors attribute changes in plasma electrolyte levels to accompanying changes in blood volume and this may be very legitimate (Bailey, 1964; Hannon et al., 1958; Spurr and Barlow, 1959).

Barbour, McKay, and Griffith (1943) proposed a widely quoted theory that the hypothalamus contains a 'water-shifting reflex' which, during cold stress, causes a centripetal shift of water into the cells. He suggested that deep hypothermia abolishes this reflex by depressing the central nervous system causing a reverse water shift and an increased extracellular fluid volume. This paper ignored the fact that the fluid shifts occurred during marked shivering (Bass and Henschel, 1956). D'Amato (1954) confirmed that the fluid shifts are a result of shivering and do not represent a primary defence against cold.

A. Packed cell volume

Packed cell volume or hematocrit is often used as an indicator of hemodilution or hemoconcentration and as an estimate of plasma and blood volume (Adolf and Molnar, 1946; Eliot, Bader, and Bass, 1949; Bass et al., 1951; Swan et al., 1953; Deb and Hart, 1956). The use of hematocrit may have some limitations as a means for calculating changes in plasma volume (Bass and Henschel, 1956). T-1824 dye experiments showed less change in blood volume than in plasma volume in humans. Swan et al. (1953) found that hematocrit gave larger calculated changes than either dye dilution calcu-

lations or plasma protein calculations. Deb and Hart (1956), using cold-exposed rats, found an increase in the absolute blood and plasma volumes (T-1824 space) with no difference in red blood cell counts or hemoglobin concentration. This was accompanied by an increased hematocrit which indicates an increased corpuscular volume. On the other hand, Chang and Shoemaker (1963), who administered labeled erythrocytes to hypothermic dogs, found a loss of red blood cells which was accompanied by a greater loss of plasma volume. This produced a relative hemoconcentration and would give a greater change than that calculated by using hematocrit as an indication of plasma volume changes.

Eliot et al. (1949) found an increased hematocrit in nude reclining men in an ambient temperature of 15.6 C. There was a very close agreement between calculated plasma volume lost and increased urine output. Hemoconcentration still occurred when cold diuresis was completely inhibited by antidiuretic hormone. Therefore, some other mechanism than diuresis must control the plasma volume. Adolf and Molnar (1946) inferred that 850 ml of water were lost from the blood partly by diuresis and partly into interstitial fluid in cold-exposed nude men. They found about a 12% increase in hematocrit when the ambient temperature dropped from 26.7 to 15.6 C. As the temperature continued to drop to 0.6 C, there was no further increase in hematocrit. D'Amato (1954) found similar results in dogs. There was a marked increase in hematocrit when dogs were cooled from control temperatures to a rectal temperature of 28 C, but further cooling the rectal temperature to 20 C had no significant additional effect on packed cell volume.

Bass et al. (1951) found that the increased hematocrit in cold-exposed men was modified with extended cold exposure.

Everett and Matson (1961), in studies with labeled erythrocytes, demonstrated a significant increase in the number of red blood cells in rats

after 24 hr of cold exposure. This was accompanied by a decreased hematocrit and an increased blood volume. After 6 weeks the total blood volume was increased 20%, with the increase in erythrocytes being greater than the increase in plasma volume. This gave an increase in hematocrit.

Hannon et al. (1958) found an increased hematocrit in cold-acclimatized rats while Baker (1960b) found an initial hemoconcentration in rats which was followed by increased blood and body water volumes after prolonged cold exposure.

Munday et al. (1958) found hemoconcentration in hypothermic rats but found hemodilution in cold-exposed rats (Munday and Blane, 1960) in a different experiment. Hemodilution also occurs in the pigeon during both short- and long-term cold stress and in the reptile during short-term cold stress (Munday and Blane, 1961).

Swan et al. (1953) found a decrease in body water and blood volume and hemoconcentration in dogs and humans during general hypothermia. Platner and Hosko (1953) found hemoconcentrations ranging from 7.4 to 16.4% for the turtle, hamster, dog, rat, and cat undergoing hypothermia.

Sheep showed a slight increase from 36.7 to 36.9% in hematocrit (not significant) when exposed to long-term cold (Hess, 1963). There was no correlation between degree-hours and hematocrit. On the other hand, Bailey (1964) found that sheep receiving a voluntary water intake showed a significant ($P < 0.01$) increase in hematocrit from 35.5 to 38.1% during short-term cold exposure. The results were similar with sheep given 1000 g of water per day intraruminally, but the differences were not significant in animals which similarly received 2000 or 3000 g of water daily.

EXPERIMENTAL

I. Objectives

A. To study stress in the sheep resulting from short-term exposure to cold as indicated by adrenal cortical function.

B. To study changes in the sheep due to short-term cold exposure, in eosinophil count and packed cell volume, and in the plasma concentrations of sodium, potassium, calcium, magnesium, and glucose.

C. To study the effect of short-term cold exposure on the jugular vein and rectal temperatures of the sheep.

II. Animals

A. Preliminary trial

Six mature, unshorn wethers weighing 72 to 102 kg and ranging from 2 to 5 years of age were used in the preliminary trial during the winter of 1964 and 1965. During the experiments, the animals were kept in the metabolism cages described in Appendix I. The sheep underwent a training period of at least 1 week at a control room temperature of 10 to 16.5 C before the trial began. The animals were fed good-quality legume hay and received water ad libitum. Cobalt-iodized salt was available at all times. Freedom of movement within the metabolic cages was the only exercise allowed.

B. Experiment

From the six mature wethers in the preliminary trial, three animals (one 4-year-old and two 3-year-olds) were selected on the basis of quietness and ease of training. To this group, one 5-year-old wether of known quietness was added. The animals weighed from 72 to 91 kilograms. The animals were shorn to a fleece length of about 5 mm just before a training period which ranged from 3 to 7 days. The short fleece length was necessary to intensify the cold effects. For the duration of the training and experimental

periods, each animal was kept in a metabolism cage inside the temperature-controlled chamber described in Appendix II. The first experimental animal was given good-quality grass-legume hay and water ad libitum. Animals two, three, and four were given good-quality pelleted grass-legume hay and water ad libitum. The animals were fed and watered daily at 8:45 AM, and the feed and water consumption was recorded (Appendix IV). Urine was collected and the total urine volume was measured daily at 8:45 AM (Appendix IV). Cobalt-iodized salt was available at all times. Freedom of movement within the metabolism cages was the only exercise allowed. Before each animal was used it was kept with other wethers in a pen in the same barn that housed the cold chamber.

III. Experimental schedule

A. Preliminary trial

Three animals were kept in metabolism cages in a heated room with a moderately fluctuating temperature of 10 to 16.5 C. After the training period, two control samples of blood were taken from each sheep. One was taken at 3:00 PM and another was taken at 9:00 AM, 2 days later. Following the 9:00 AM sampling the sheep were transferred from the cages in the heated room to metabolism cages in the cold alley of the barn which had a temperature range of -3 to 4 C. Samples of blood were subsequently taken at 12:00 M, 3:00 PM, and 9:00 PM that day; 12:00 M on the following day; and 12:00 M on the day following. A total of two control and five treatment samples were obtained from each sheep. This schedule was then repeated for the other group of three sheep.

Rectal temperatures and wet and dry bulb ambient temperatures were recorded periodically.

The sampling schedule had to be carried out when the weather had settled into a steady cold spell so as to have low temperatures in the alley

of the barn.

B. Experiment

1) General

The size of the cold chamber limited the project in that only one animal could be used at a time and each animal was kept in the chamber for the duration of the experiment.

After each animal was trained and handled in the chamber for 3 to 7 days, the sampling schedule was begun. It consisted of a control period of 3 days, followed by a cold period of 3 days, and then a rewarmed period of 3 days. Fourteen samples of blood were obtained during each period to give a total of 42 samples from each sheep over the 9 days of sampling. The schedule was repeated for each of the four sheep.

Wet and dry bulb and rectal temperature readings were taken immediately before each sample of blood was taken.

Jugular vein temperature readings were obtained just prior to each sampling time for the last two sheep in the experiment.

2) Control period

The temperature in the chamber was maintained at 20.6 $\pm$ 1.0 C.* On day 1, samples of blood were taken at 9:00 AM, 10:30 AM, 12:00 M, 1:30 PM, 3:00 PM, 4:30 PM, 6:00 PM, 7:30 PM, and 9:00 PM for a total of nine samples.

On day 2, samples were taken at 9:00 AM, 3:00 PM, and 9:00 PM.

On day 3, samples were taken at 9:00 AM and 3:00 PM.

3) Cold period

Sampling times were the same as for the control period. Following the 9:00 AM sampling on the 1st day of the cold period, the temperature in the chamber was lowered over a period of 3 hr to a temperature of 1.3 $\pm$ 1.5 C

*Average temperature $\pm$ one standard deviation.

for the remainder of the cold period. Half-way through the cold period the temperature was raised 10 C or less for about 15 min while the cooler coils were defrosted.

4) Rewarmed period

Sampling times were the same as for the control period. Following the 9:00 AM sampling on the 1st day of the rewarmed period, the temperature in the chamber was allowed to increase over a period of 3 hr to a temperature of 19.2 $\pm$ 2.0 C where it was maintained by adjusting the door opening.

IV. Temperature measurements

A. Air temperature

The temperature within the chamber was recorded from wet and dry-bulb thermometers. The per cent relative humidity was calculated from these readings using the relative humidity table in Hodgman, Weast, and Selby (1960).

B. Rectal temperature

The procedure followed was that used by Veeraraghavan (1963). Rectal temperatures were measured with a 'Banjo-type' thermistor probe (time constant, 0.8 sec) connected to a direct reading bridge thermistor instrument (YSI 12-channel tele-thermometer[1]). The depth of insertion was 10 cm and the probe was held until a constant reading was obtained. The 'Banjo' probe was previously calibrated with a mercury thermometer and found to have precision and linearity over the normal range of body temperatures.

C. Jugular vein temperature

The procedure followed was that used by Veeraraghavan (1963). Jugular vein temperatures were measured with a thermistor probe (time constant, 0.6 sec) embedded in the tip of a 22-gauge hypodermic needle 10 cm in length which was connected to the YSI tele-thermometer. The temperatures were

[1]Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio, USA.

recorded by inserting the hypodermic probe in either the left or right jugular vein in the mid-neck region. The sudden rise of the indicator needle on the tele-thermometer confirmed the entry of the probe into the jugular vein after it had passed through the skin. The probe was carefully held in this position until a constant reading was obtained. The readings were then corrected from a conversion chart previously prepared by comparison of readings obtained with the hypodermic needle to those obtained with the 'Banjo' probe. The conversion chart gave a linear relationship between the two probes over the short temperature range measured (Appendix V).

V. Sampling of blood

Blood samples of about 10 ml were obtained from either the right or left jugular vein using a B-D Vacutainer assembly[2] fitted with a 1.5 inch 20-gauge needle. After the sample was obtained it was inverted slowly at least five times to ensure mixing with the anticoagulant. The stoppered vacutainer sample tubes contained 0.2 cc of Heparin Sodium, USP[3] (1000 USP heparin units/cc) and were evacuated to a negative pressure of 700 mm of mercury.

VI. Analytical procedures

A. Hematocrit or packed cell volume

Within 5 min after drawing each blood sample, duplicate aliquots of blood were drawn into micro-capillary tubes which were then sealed with modelling clay. These micro-capillary tubes were centrifuged in an IEC micro-capillary centrifuge[4] for 15 min at 11,500 revolutions per minute. The per cent packed cell volume was then read directly from an IEC micro-

[2]Becton, Dickinson and Co., Rutherford, New Jersey, USA.
[3]Eli Lilly and Co., Indianapolis, Indiana, USA.
[4]International Equipment Co., Boston, Massachusetts, USA.

capillary reader[5] and recorded.

B. Eosinophil counting

While the hematocrit samples were being centrifuged, eosinophil counts were made. The procedure followed was that used by Speirs and Meyer (1949), with slight modification. A 2-cc syringe with a short rigid piece of plastic tubing attached to the Luer-lok connection served as an aspirator for filling the pipets.

Blood was drawn into the white blood cell diluting pipet until the 0.5 mark was reached. The diluent (Appendix VI) was then drawn into the pipet until the 11 mark was reached. The pipet was shaken by hand at right angles to the capillary stem for 60 sec, the first 5 drops were discarded, and then both sides of the counting chamber[6] were filled carefully. The chamber was allowed to stand for several minutes and then each chamber was counted under a microscope with a 10X ocular and a 10X objective lens. All 16 squares were counted in each chamber. The results were divided by two to find the average and multiplied by a factor of 6.25 to obtain the number of eosinophils per sample (Appendix VI).

The eosinophils are easily recognizable as distinct pink bodies of granules within the cell that stand against a colorless background. The granules may be in a central mass or scattered around the periphery of the cell. The nucleus is completely colorless, sometimes resulting in granules being arranged around the invisible nucleus in a quarter-moon configuration.

C. Plasma samples

After the removal of aliquots for hematocrit determination and eosinophil counting, the remaining whole blood was centrifuged at about 2500 rpm for 10 to 12 minutes. The plasma was drawn off and put in small,

[5] International Equipment Co., Boston, Massachusetts, USA.

[6] Fuchs-Rosenthal Chamber, 0.2 mm deep, C. A. Hausser and Son, Philadelphia, Pennsylvania, USA.

capped, plastic capsules. The plasma samples were kept cool for a few hours until they could be brought into the laboratory where they were quickly frozen and stored for later analyses for sodium, potassium, calcium, magnesium, and glucose.

D. The determination of sodium, potassium, and calcium by flame photometry

The procedure used, with slight modifications, was developed by Kingsley and Schaffert (1953). A Beckman DU quartz spectrophotometer[7] with a flame photometer attachment and a photomultiplier tube incorporated into the spectrophotometer was used for the determinations.

Details of the preparation of reagents and the derivation of formulas are found in Appendix VI.

The flame photometer was equipped with an oxygen-hydrogen atomizer. The hydrogen fuel pressure was set at 5 psi and the oxygen pressure at 16 psi for the sodium and potassium analyses. For the calcium analysis, the oxygen pressure was reduced to 11 psi and the hydrogen pressure increased to 6 psi to obtain a greater sensitivity. The flame photometer was started and adjusted in accordance with the operating manual supplied[7].

Samples and standards were prepared by adding 0.2 ml of plasma or standard with a 'pipet calibrated to contain' to 10 ml of 0.02% Sterox[8] in a test tube. The pipet was filled and washed out three times with the Sterox solution. The solution was then mixed well.

1) Sodium

The settings used for sodium determination were: wavelength, 589 mμ; slit width, 0.02 mm; spectrophotometer sensitivity, five turns clockwise; power supply sensitivity, position six; resistance switch, position two;

[7] Beckman Instruments Inc., Fullerton, California, USA.

[8] Sterox, S.E., Monsanto Chemical Co., St. Louis, Missouri, USA.

phototube knob, out, to position photomultiplier tube.

With the blank (0.02% Sterox) in position, the phototube switch on, and the transmittance scale set at 0% T, the null point needle was adjusted to zero by adjusting the dark current knob. A 150 meq/1 sodium standard was then placed in position and with the transmittance scale set at 75% T the null point needle was adjusted to zero by adjusting the sensitivity knob. Then 125 and 100 meq/1 sodium standards were read by placing them in position and setting the null point needle to zero via the transmittance knob. If the 100 meq/1 standard did not read approximately 62.5% T, the procedure was repeated. If it did read approximately 62.5% T, the unknowns were read in the same manner as the two less-concentrated standards. The blank was used after every third sample to reset the 0% T reading and the dark current was adjusted accordingly. At the end of every set of unknowns the 150 meq/1 sodium standard was again read. If the reading differed from 75% T by more than 1% T, the results were discarded and the samples were reread. Each sample was done in duplicate and each duplicate was read twice. These four readings were averaged for each sample and the sodium concentration was obtained by means of the following formula:

$$(4 \times \text{unknown reading}) - 150 = \text{meq/1 of sodium}$$

2) Potassium

The settings used for potassium determination were: wavelength, 770 mμ; slit width, 0.5 mm; spectrophotometer sensitivity, five turns clockwise; power supply sensitivity, position nine; resistance switch, position three; phototube knob, in, to position red phototube.

The procedure was similar to that used for sodium except that the most concentrated standard was set at 50% T.

The potassium concentration was obtained by inserting the mean value of the four readings for each sample into the following formula:

$$\frac{\text{unknown reading}}{10} = \text{meq/l of potassium}$$

3) Calcium

The settings used for calcium determinations were: wavelength, 422.7 mμ; slit width, 0.05 mm; spectrophotometer sensitivity, two turns clockwise; power supply sensitivity, position eight; resistance switch, position two; phototube knob, out, to position photomultiplier tube.

The procedure was then continued as for sodium, except that the most concentrated standard was set at 50% T.

The calcium concentration was obtained by inserting the mean value of the four readings for each sample into the following formula:

$$\frac{\text{unknown reading}}{10} = \text{meq/l of calcium}$$

E. The volumetric determination of calcium and magnesium

The method of Carr and Frank (1956), which involves titration with ethylenediamine tetraacetate (EDTA) to an endpoint indicated by eriochrome black T (EBT), was used with slight modification. The technique allows the titration of the calcium and magnesium without first precipitating the protein.

The reagents were prepared as indicated in Appendix VI.

All of the determinations were done in duplicate.

Either 1.0 ml of mixed standard or 1.0 ml of plasma were placed in 12-ml graduated centrifuge tubes and 2.5 ml of deionized water were added, followed by 0.5 ml of the saturated solution of ammonium oxalate. The contents were mixed well using a Vortex test tube mixer[9] and the tubes

[9] Vortex Jr. Mixer, Scientific Industries Inc., Springfield, Massachusetts, USA.

were capped with parafilm. The tubes were then allowed to stand overnight to allow complete precipitation.

The tubes were next centrifuged for 15 min at 900 x g and the supernatant solutions were decanted off into labeled test tubes for the analysis of magnesium. The centrifuge tubes containing the precipitated calcium oxalate were inverted for 5 min on filter paper.

1) Calcium

Using a fine spray from a wash bottle, the precipitated calcium oxalate was broken up with about 5 ml of the dilute solution of ammonium hydroxide. The contents were mixed on the Vortex mixer and were then centrifuged for 12 min at 900 x g. The supernatant solution was decanted off and discarded. The centrifuge tubes were tapped with a finger to loosen the washed precipitate.

The precipitate was then dissolved with 1.0 ml of the perchloric acid solution and 3.0 ml of the ethanolamine-magnesium-EDTA complex were added. The samples were titrated within 5 min in order to avoid the reprecipitation of calcium oxalate.

Two or three drops of EBT indicator were added to each tube and the calcium was determined by titration, with the working solution of EDTA for the analysis of calcium delivered from a 10-ml buret. The end point is a clear, sky-blue color. All the tubes in each set were brought to the same end-point color obtained for the standard. Thorough mixing was ensured by using the Vortex mixer during titration.

The calcium concentration was then calculated from the average of the duplicates by the formula:

$$\frac{\text{sample titration x standard Ca}}{\text{standard titration}} = \text{meq/l of Ca}$$

2) Magnesium

Initially, a 3.0-ml aliquot of the supernatant fluid collected earlier was put into a 50-ml Erlenmeyer flask for titration. With such a small volume, the end point was difficult to see, so in later analyses the 3.0-ml aliquot was put into a 10-ml test tube and mixed by means of the Vortex mixer during titration. This enabled a much easier determination of the end point.

In either case, 3.0 ml of a solution of ethanolamine and two or three drops of the solution of EBT were added to the 3.0-ml aliquot of supernatant fluid. With the use of a 10-ml buret, the magnesium was titrated immediately with the working solution of EDTA for the analysis of magnesium. The end point was similar to that for the titration of calcium. However, in the magnesium determination, care had to be taken to avoid over-titration, as the color was slower developing than was the case for calcium.

The magnesium concentration was then calculated from the average of the duplicates by the formula:

$$\frac{\text{sample titration x standard Mg}}{\text{standard titration}} = \text{meq/l of Mg}$$

F. The determination of plasma glucose

Plasma glucose concentrations were determined through the use of a Technicon Auto Analyzer[10]. The procedure measures the decolorization of potassium ferricyanide in the potassium ferricyanide-potassium ferrocyanide oxidation reduction reaction. The color was measured at 420 mμ using a flow cuvette with a 15-mm light path according to the micro-glucose procedure supplied with the Auto Analyzer.

The plasma was diluted 0.5 ml to 3.33 ml for a dilution of 1:6.67

[10]Technicon Instruments Corporation, Chauncey, New York, USA.

using an automatic dilutor[11]. The determination was then made with the Auto Analyzer running at the rate of 60 determinations per hour. Each sample was prepared in duplicate and each duplicate was run through twice to provide four readings on each sample.

Each peak was then read on an Auto Analyzer N2 blood glucose chart reader and an average value determined for each sample. This value was then multiplied by 2/3 because the standards supplied by Technicon correspond to a 1:10 dilution of the sample.

G. Statistical analysis

Where applicable, the analysis of variance was used to assist in interpreting data. This was done using program BMD02V (analysis of variance and factorial design) and the IBM 7040 in the Department of Computing Science, University of Alberta, Edmonton, Alberta.

The data were further analyzed through the use of the F test and Duncan's new multiple range test (Steel and Torrie, 1960). Sheep, temperature treatment, and sampling time were considered as fixed effects.

The t test was used to measure the precision of the two methods for doing calcium determinations (Appendix VII).

A program was designed to make use of the IBM 7040 for the conversion of the data to a relative basis which would facilitate graphical expression of the results. The actual values of the various parameters studied are recorded in Appendix III.

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[11]Automatic dilutor, Labindustries, Berkeley, California, USA.

RESULTS, DISCUSSION, AND SUMMARY

I. Preliminary trial

A. Results and discussion

Transferring the sheep from the heated room (10 to 16.5 C) to the cold alley of the barn (-3 to 4 C) resulted in a rapid decrease in the number of circulating eosinophils (Fig. 1) with a minimum count being obtained 6 hr after the beginning of cold exposure. At the 6-hr sampling time the eosinophil count had decreased 26% from a control value of 786/mm^3. After 27 hr of cold exposure the eosinophil count had increased again to a maximum level which was 27% greater than the control value.

The rapid decrease in circulating eosinophils suggests adrenal cortical hyperactivity due to stress (Speirs and Meyer, 1949; Miller, 1955). In this respect, Weeth et al. (1959) have reported that a combination of cold and fasting produced eosinopenia in the sheep.

After 6 hr of cold exposure, both plasma potassium and plasma sodium had increased about 6% (Fig. 1) over the respective control values of 4.65 and 143.9 meq/liter. The sodium concentration then returned to its control value and remained near that level for the remainder of the cold period. On the other hand, potassium concentration remained 3 to 8% higher than the control value, except for the last sample taken during the period of cold.

The fact that plasma sodium concentration increased as the eosinophil count decreased and then reversed its direction of change when the eosinophil count started to return to normal (Fig. 1) is suggestive of increased minerocorticoid production during the period of adrenal cortical hyperactivity. Such a conclusion is supported by the suggestion by Munday and Blane (1961) that increased minerocorticoid production occurs during the early stages of cold exposure.

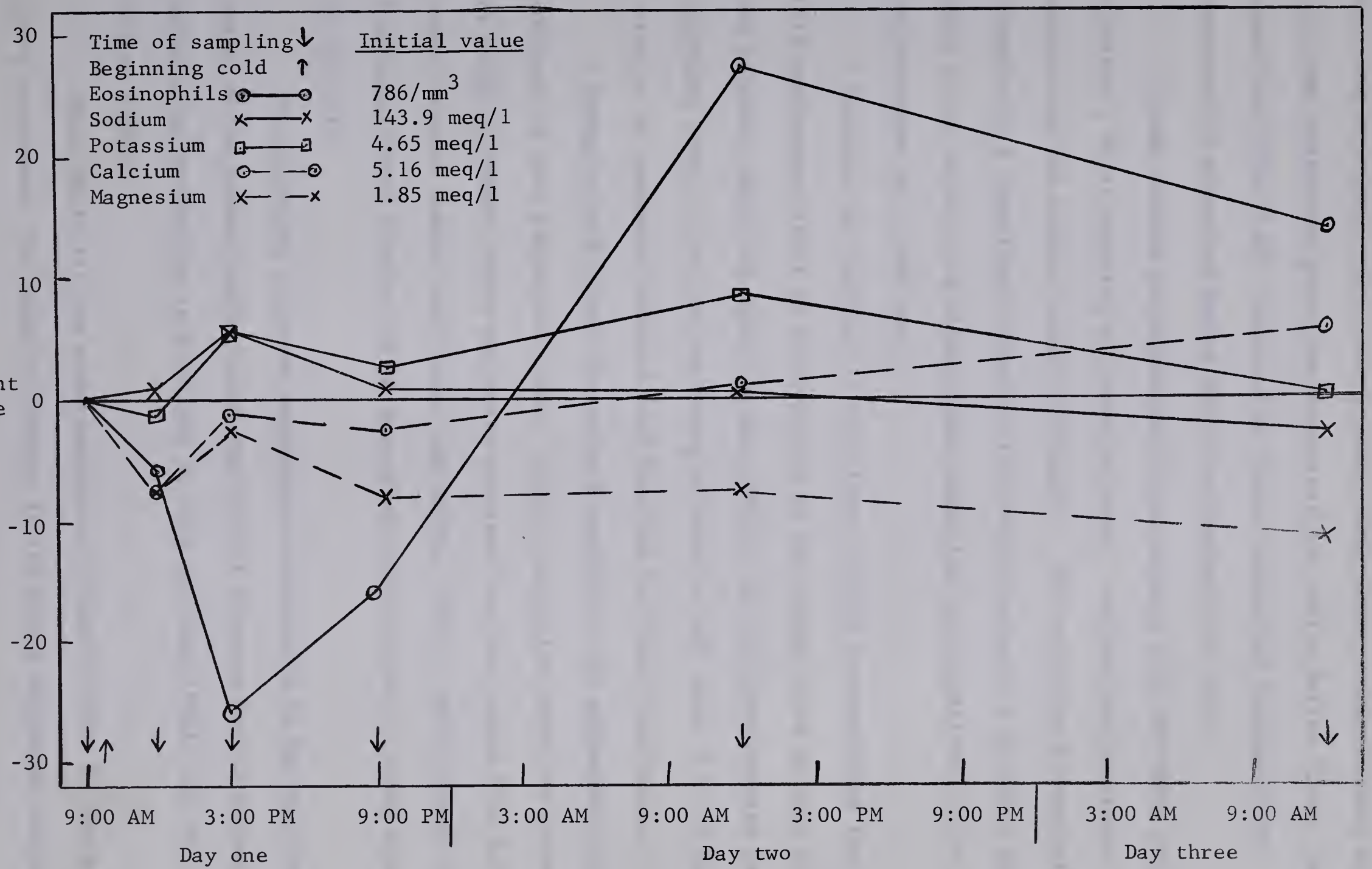
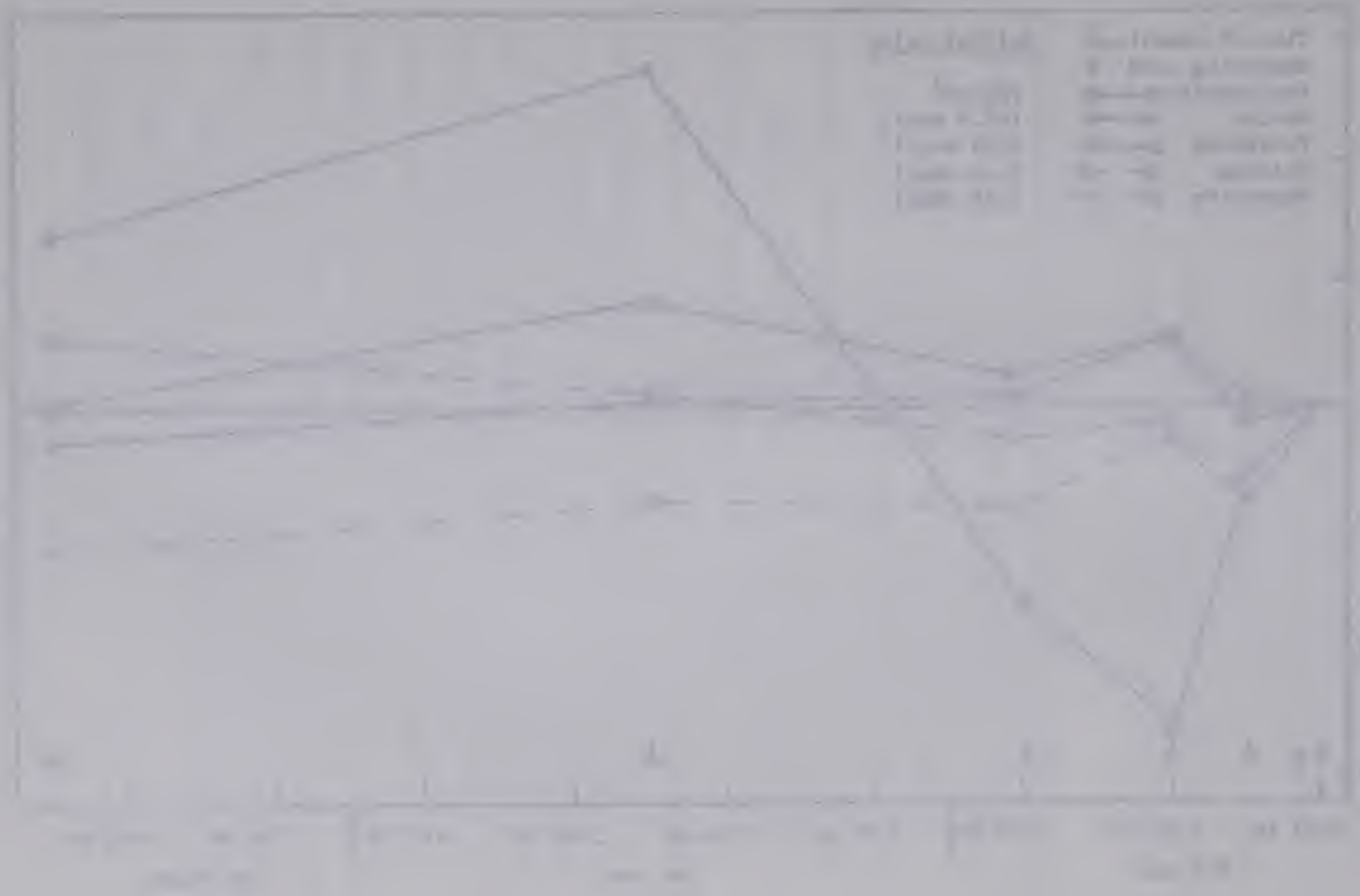


Fig. 1. Effect of cold exposure on blood eosinophil counts, plasma sodium, plasma potassium, plasma calcium, and plasma magnesium



The increases in plasma sodium and potassium are in agreement with work done by others using sheep. Hess (1963) found nonsignificant increases in plasma sodium and potassium concentrations, while Bailey (1964) found significant ($P < 0.05$) increases in plasma sodium and nonsignificant increases in potassium during short-term exposure to cold.

Plasma calcium and magnesium concentrations both decreased about 6% after 3 hr of exposing the sheep to cold. Calcium then increased, approaching the control value (5.16 meq/1). The magnesium concentration showed only a transitory return to its control value of 1.85 meq/1 and after 51 hr in the cold alley plasma magnesium concentrations of the sheep had decreased by 11 per cent.

Although the decrease in the plasma calcium concentration found in this preliminary trial is the opposite to the change noted in rats (Elliott and Crismon, 1947) and sheep (Bailey, 1964), the calcium:potassium ratio decreased from 1.11 at the beginning of cold to 1.05 after 3 hr of cold, which is in agreement with the work reported by Elliott and Crismon (1947).

Normally cold stress causes an elevation in the magnesium concentrations of both plasma and urine. Urinary magnesium excretion increases in order to remove excess magnesium released into the plasma from peripheral tissues during cold (Heagy and Burton, 1947). Bailey (1964) found a nonsignificant rise in plasma magnesium concentration in sheep exposed to the cold.

The decreases in plasma magnesium concentration in the preliminary trial of the present project show the typical decrease due to increased aldosterone production as described by Care and Ross (1963) and Scott and Dobson (1965).

Thus, while all the other parameters indicate stress in the preliminary experiment, the observed changes in the plasma magnesium concentrations

are not those typically expected due to cold stress. Therefore, it seemed apparent that the stressor agent causing the adrenal hyperactivity may not have been cold.

Reid and Mills (1962) showed that the emotional stress which occurred when sheep were moved about or changed from their regular training program caused abrupt increases in cortisol levels within 2 to 4 hr of the handling. This occurred even when the sheep showed no visible signs of stress. In later work, Reid (1962) found that cold stress also caused an increase in plasma cortisol but that this increase was delayed several hours longer than when the sheep were subjected to emotional stress. Cold caused a more prolonged high level of plasma cortisol than did emotional stress.

In the preliminary trial of the present study the sheep were moved from metabolism cages in the warm room into metabolism cages in the cold room. The animals showed considerable excitement at this transfer. It appeared, then, that a more discriminating trial was necessary to see if the stress indicated by the observed eosinophil and electrolyte changes was emotional or cold induced.

B. Summary

1) Eosinophil counts decreased rapidly in the sheep during the first 6 hr of cold exposure but returned to normal after 12 to 27 hr in the cold.

2) The plasma sodium and potassium concentrations increased during the first 6 hr of cold exposure and then approached the control values after 12 hr of cold.

3) The plasma calcium concentration showed a transitory decrease during the first 6 hr of cold exposure.

4) The plasma magnesium concentration showed a slow but marked decrease throughout the 51-hr cold period.

5) The parameters studied indicated that the sheep were subjected to stress in the cold period. The stress, however, may have been emotional rather than due to the cold.

II. Experiment

A. General responses of the animals to cold

After 3 to 4 hr of cold exposure (1.3 $\pm$ 1.5 C) visible shivering began. Shivering continued throughout the cold period except in the case of one animal which stopped visible shivering during the last 24 hr of cold exposure. All of the sheep appeared extremely uncomfortable in the cold, and huddled in the cage when resting.

Feed consumption was depressed during the first 24 hr of cold exposure but then increased again to the control level after 3 days in the cold. There was a significant ($P < 0.05$) difference in the consumption of pellets between individual sheep. There were no significant differences between the average daily feed consumed in the three periods. These data are recorded and analyzed in Appendix IV, Tables 1 and 2.

Average daily water consumption was significantly higher ($P < 0.05$) during the rewarming period than during the cold period, but the average daily water intakes during both of these periods were not significantly different from those observed during the control period (Appendix IV, Table 2).

Average daily urine volume was not significantly changed by cold exposure (Appendix IV, Table 2).

B. Body temperature

1) Results and discussion

Cold exposure significantly ($P < 0.01$, see Appendix III, Table 2) lowered the mean rectal temperature from 39.0 to 38.8 C (Appendix III, Table 5). During the rewarm period the mean rectal temperature was significantly ($P < 0.01$) increased to 38.9 C.

The rectal temperature did not show an appreciable fall until after the first 12 hr of cold exposure (Fig. 2 and Table 1) and it then remained depressed for the remainder of the cold period. After 5 hr during the rewarm period, the rectal temperatures returned to normal.

The mean jugular vein temperatures were decreased significantly ($P < 0.05$) in the cold from 38.5 to 38.4 C (Appendix III, Tables 2 and 5) and subsequently increased again to 38.5 C in the rewarming period (significant, $P < 0.05$).

The jugular vein temperature decreased rapidly during cold exposure and returned to the control value 12 hr after cold exposure began where they remained constant thereafter except for a transitory decrease at 48 hr cold exposure (Fig. 3 and Table 1).

The lag indicated by measurement of rectal temperatures when compared to jugular vein temperatures is comparable to that found by Veeraraghavan (1963).

The body temperatures reported are within the range of 38.3 to 39.9 C reported for sheep by Dukes (1955) and compare favorably with those of Bailey (1964), Hess (1963), and Veeraraghavan (1963).

Veeraraghavan (1963), who studied sheep kept at an ambient temperature of 23 to 27 C, found the rectal temperature to be about 0.2 C lower than the jugular vein temperature in the same animals. However, in the present study, jugular vein temperatures were 0.4 to 0.5 C lower than rectal temperatures. The latter results agree with those shown for the dog by Horvath, Rubin, and Foltz (1950).

Table 1. Mean rectal[a] and jugular vein[b] temperatures (C)

	Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Rectal	39.0	39.0	38.9	39.0	39.0	39.0	39.0	39.0	39.0	38.9	39.0	39.1	38.9	39.0
	SE	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1
	Jugular	38.5	38.7	38.5	38.5	38.4	38.5	38.6	38.5	38.5	38.4	38.5	38.5	38.5	38.6
	SE	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.4	0.3
Cold	Rectal	38.9	38.9	38.8	38.8	38.9	38.9	38.9	38.9	38.9	38.6	38.7	38.7	38.6	38.6
	SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Jugular	38.6	38.4	38.2	38.2	38.3	38.4	38.3	38.4	38.5	38.5	38.4	38.4	38.2	38.5
	SE	0.0	0.0	0.3	0.4	0.2	0.2	0.5	0.5	0.4	0.5	0.5	0.2	0.4	0.1
Rewarm	Rectal	38.7	38.7	38.8	38.8	38.9	38.9	38.9	39.0	38.9	39.0	38.9	39.0	38.9	38.9
	SE	0.1	0.0	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2
	Jugular	38.5	38.6	38.6	38.4	38.4	38.5	38.6	38.5	38.5	38.3	38.5	38.5	38.5	38.6
	SE	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.0	0.1	0.0

[a] Four sheep.

[b] Two sheep.

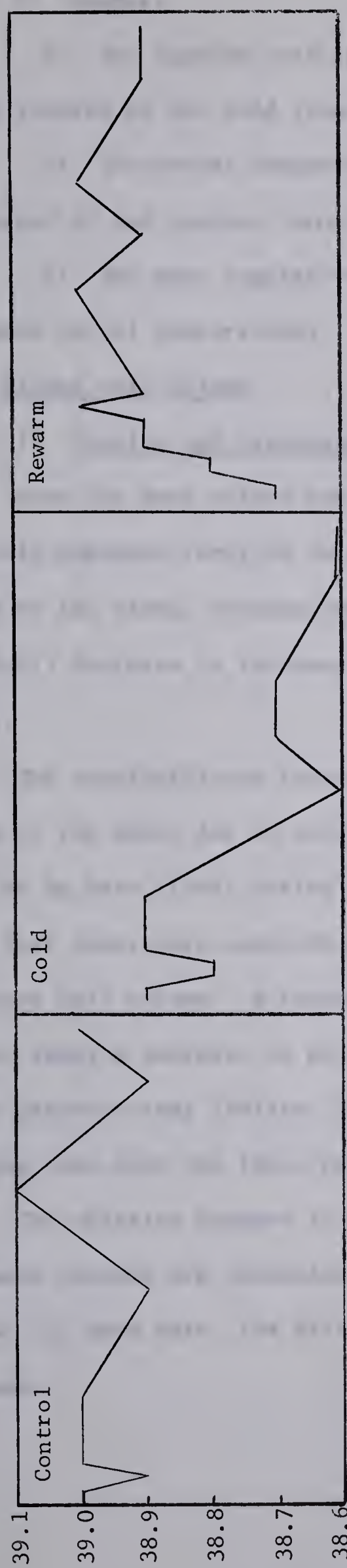
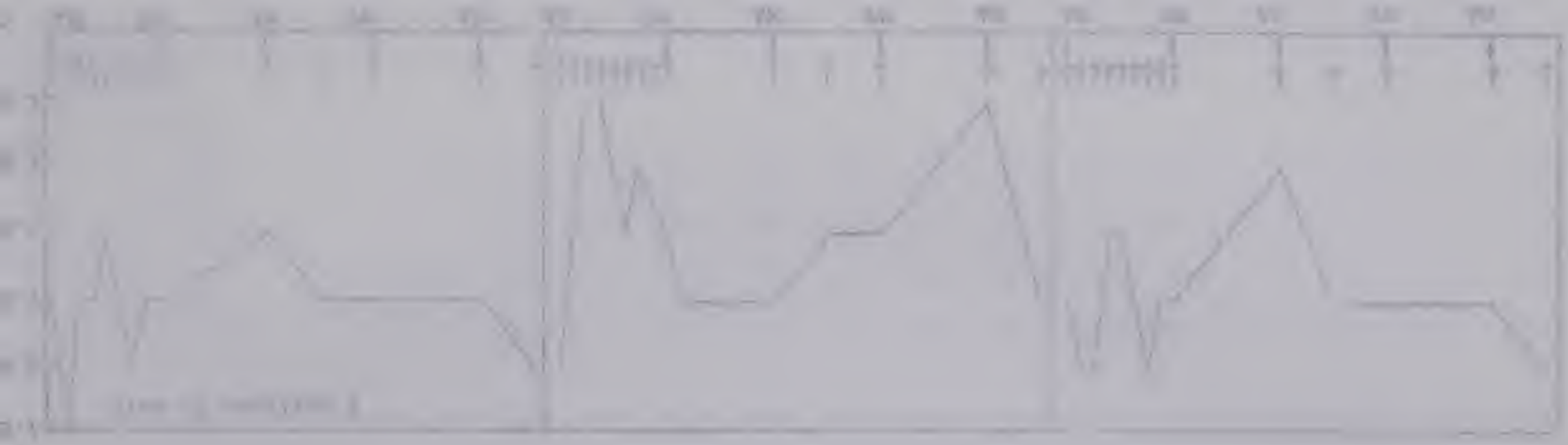


Fig. 2. Effect of ambient temperature on rectal temperature

Fig. 3. Effect of ambient temperature on jugular vein temperature



2) Summary

a) The jugular vein and rectal temperatures were both significantly lowered by the cold treatment.

b) The rectal temperatures showed a thermal lag when compared to changes in the jugular vein temperatures.

c) The mean jugular vein temperatures were 0.4 to 0.5 C lower than mean rectal temperatures.

C. Packed cell volume

1) Results and discussion

When the mean values for each treatment were compared it was found that cold exposure resulted in a nonsignificant increase in the packed cell volume of the blood, whereas rewarming the environment caused a significant ($P < 0.01$) decrease in the mean packed cell volume (Appendix III, Tables 3 and 5).

The nonsignificant increase from 34.0 to 34.4% in mean packed cell volume in the sheep due to cold is in agreement with the observations reported by Hess (1963) during long-term cold exposure. Bailey (1964) found that short-term exposure of the sheep to cold caused a larger increase in packed cell volume. A rewarming period following 1 week of cold exposure brought about a decrease in packed cell volume similar to the result reported in the present study (Bailey, 1964). The mean packed cell volumes for each sampling time over the three periods are shown in Appendix III, Table 6.

The relative changes in packed cell volume with time in the three treatment periods are tabulated in Table 2 and are shown graphically in Fig. 4. In these data, the first value in each period has been set at 100 per cent.

Table 2. Mean[a] per cent changes in packed cell volume

Samples	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	100.0	101.1	97.0	96.0	96.3	94.7	97.9	96.7	92.7	93.0	91.4	92.9	90.1	90.0
SE	0.0	0.6	2.3	1.8	2.8	2.1	2.0	3.3	2.5	3.8	3.9	3.1	3.6	4.1
Cold	100.0	99.2	102.2	106.2	106.3	102.6	105.7	106.8	103.1	106.7	110.0	106.3	105.5	107.1
SE	0.0	1.9	2.0	1.6	2.1	2.0	3.7	2.3	2.5	3.3	2.9	3.1	3.2	3.5
Rewarm	100.0	94.6	93.3	92.9	91.6	91.6	89.6	88.7	87.9	89.1	86.4	84.3	84.2	84.0
SE	0.0	0.5	1.1	1.4	1.7	2.8	2.0	2.8	1.2	2.0	1.9	1.5	2.1	1.6

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 35.9%.
First cold sample = 32.8%.
First rewarm sample = 35.5%.

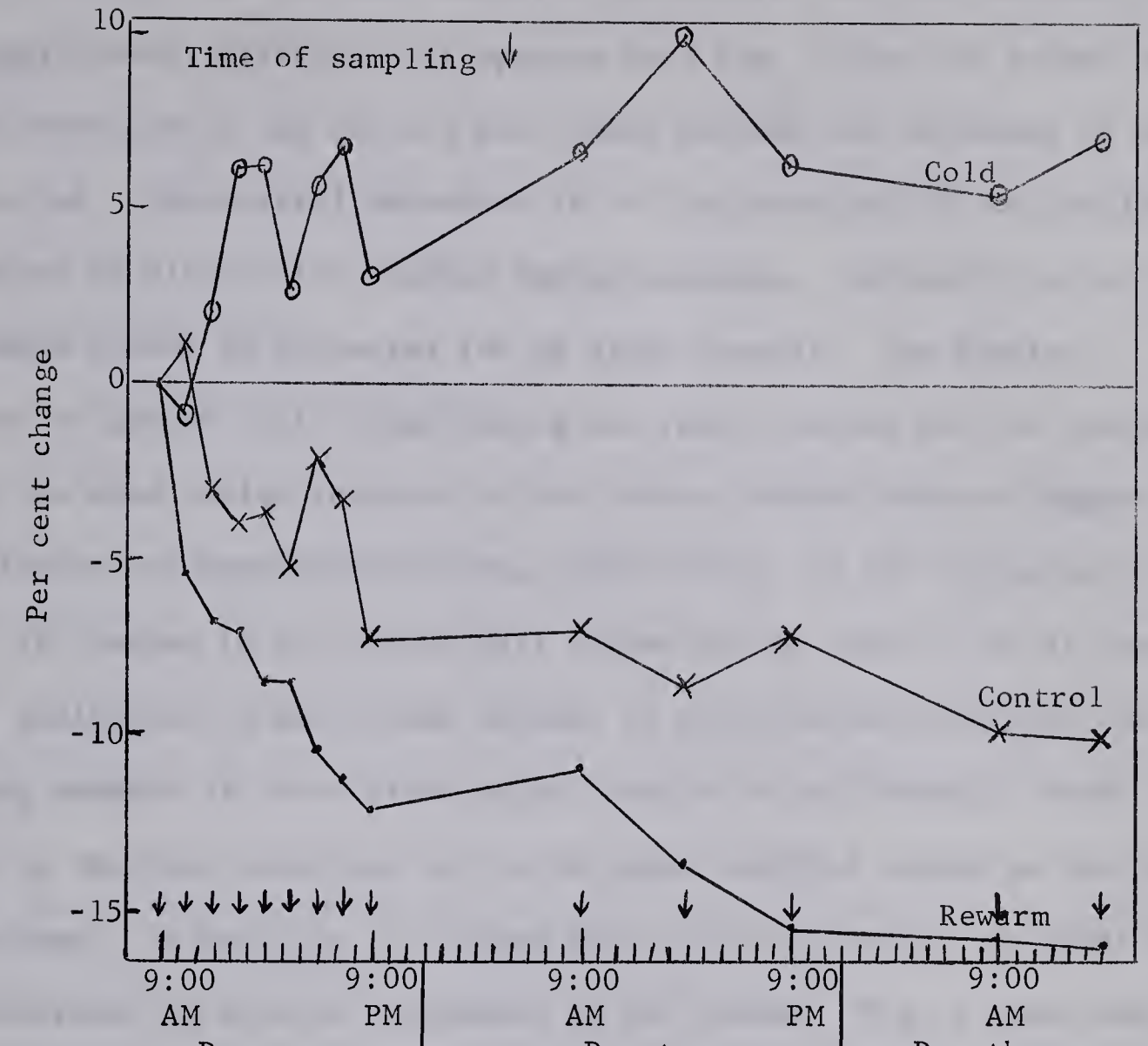


Fig. 4. Effect of temperature on packed cell volume

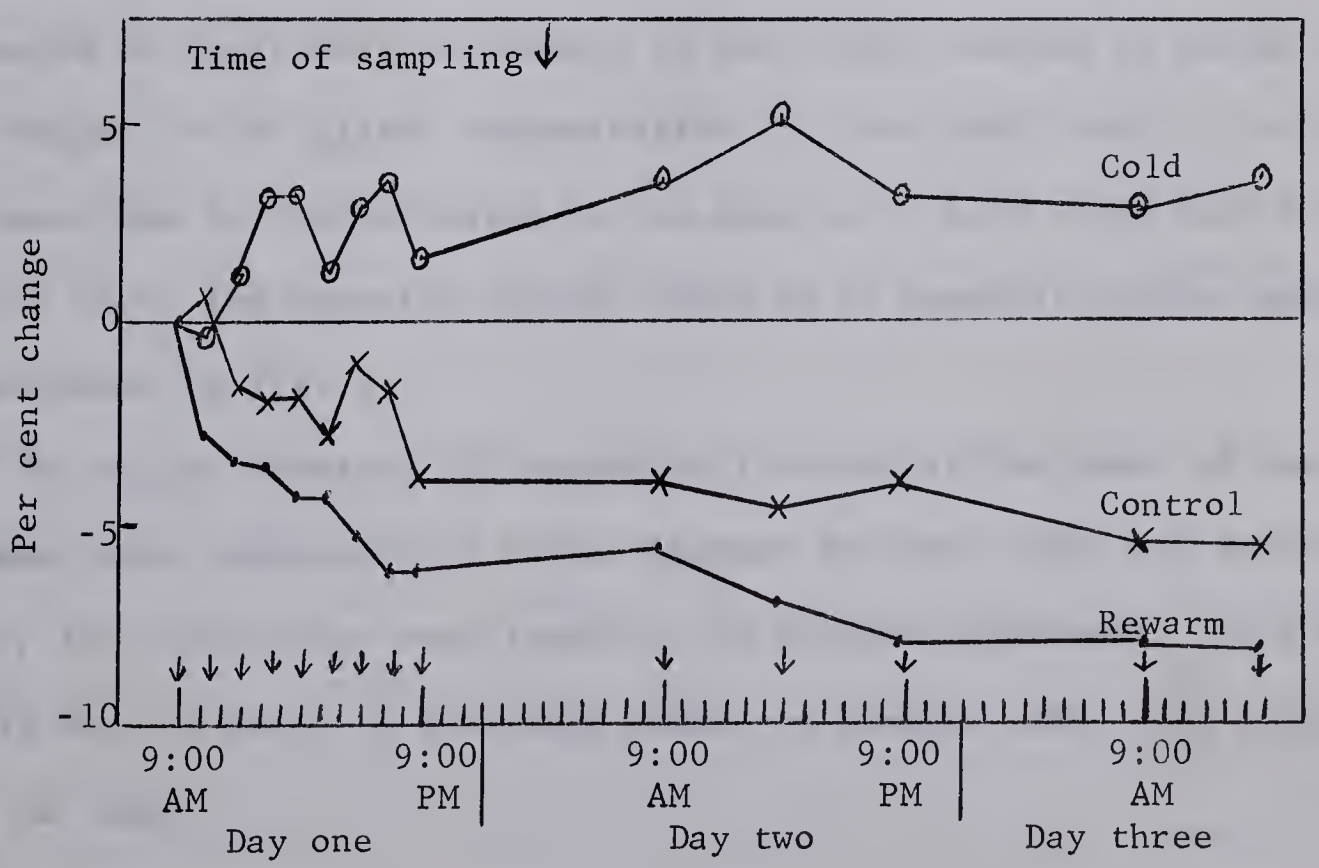
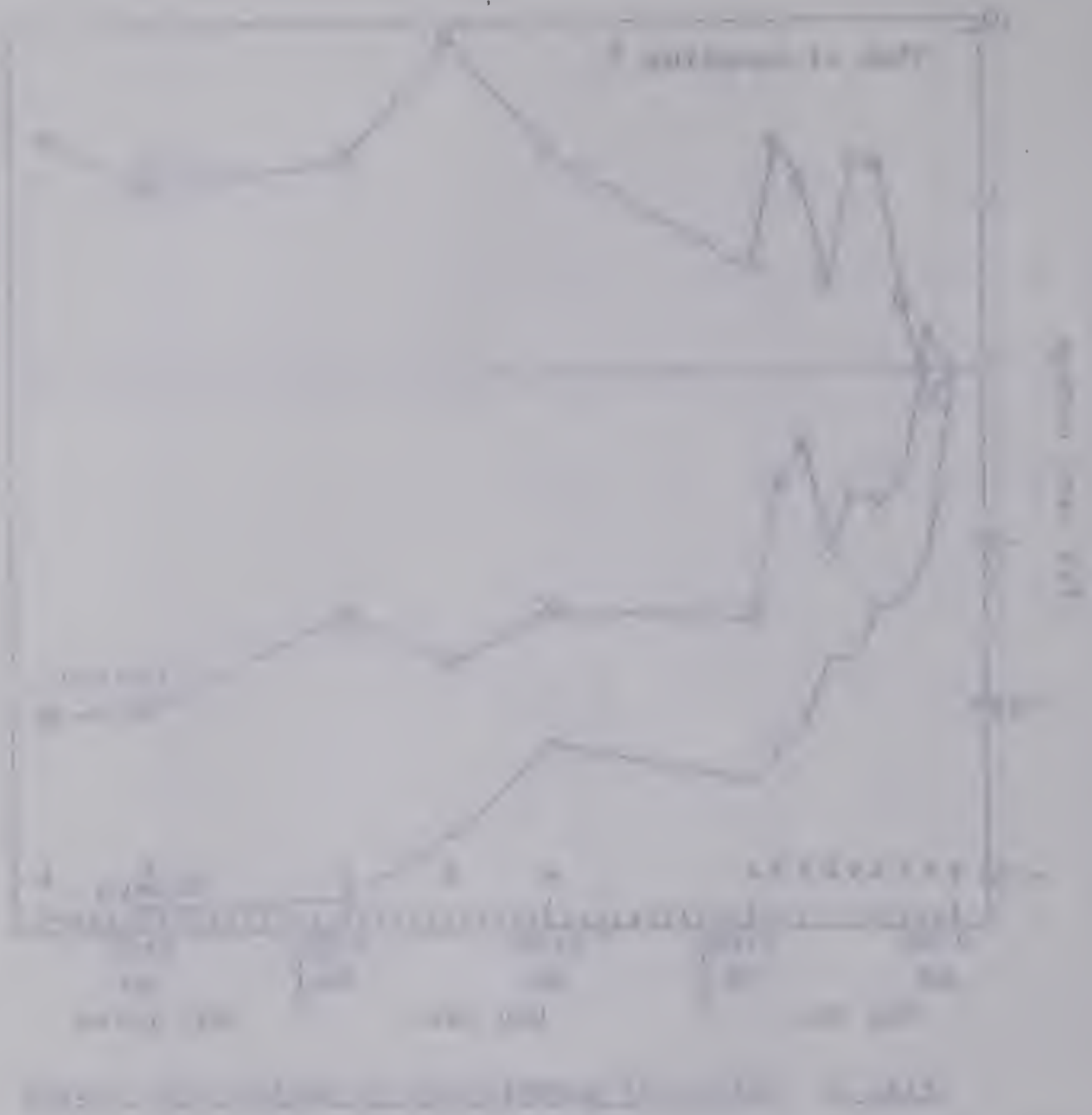
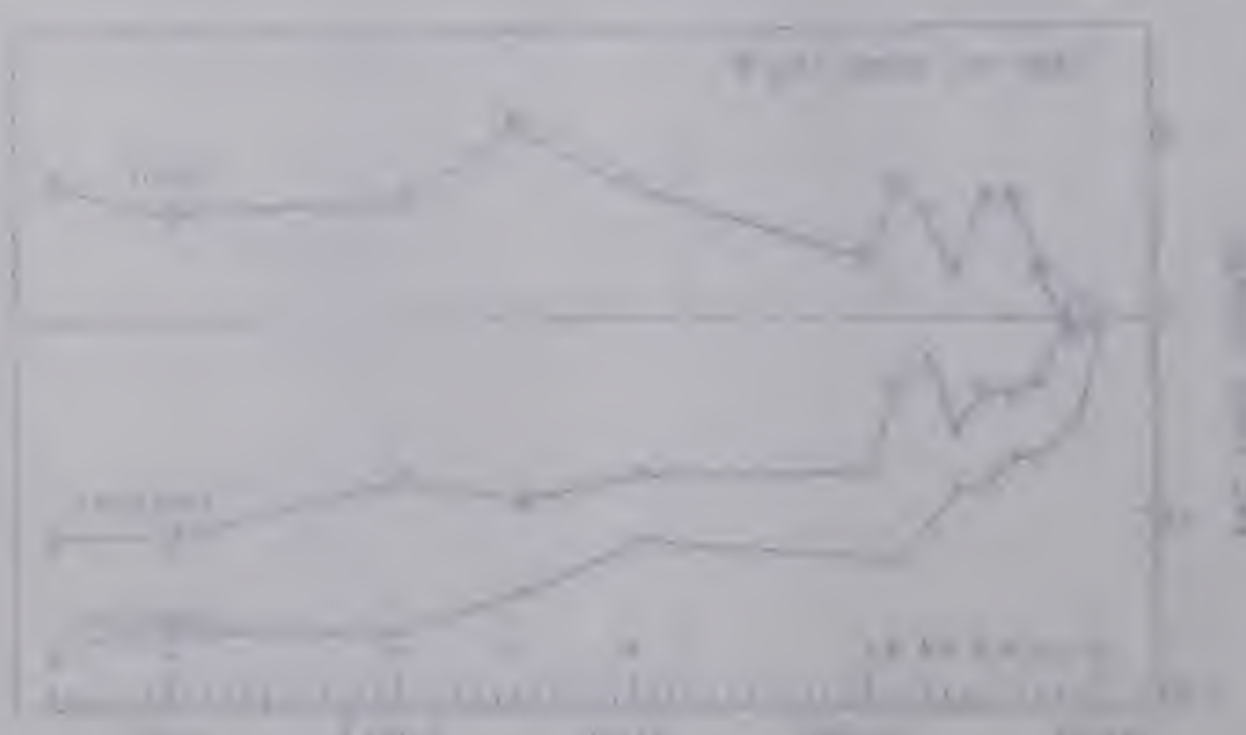


Fig. 5. Expected effect of packed cell volume changes on the concentration of plasma components





Although the treatment means for the control and cold periods were not significantly different, it appears from Fig. 4 that the packed cell volume decreased in the control and rewarm periods but increased in the cold period. The initial decreases in the two warm periods may reflect the amount of blood cells removed during sampling. Certainly all of the change cannot be accounted for by blood removal. The greater decrease in packed cell volume during the rewarm period and the increase during the cold period relative to the control period seem to suggest hemodilution and hemoconcentration, respectively, in the two periods.

If changes in the packed cell volume can be taken to be at least partly indicative of the plasma volume, it could be expected that the apparent numbers of other blood cells, such as of eosinophils, would change in the same direction and to the same relative extent as the packed cell volume. In addition, as packed cell volume increased, so might the concentrations of soluble components in the plasma. Fig. 5 shows the expected relative change in the concentration of a soluble plasma component if the change was solely due to hemodilution or hemoconcentration as suggested by packed cell volume. The effects indicated in Fig. 5 would be expected to apply most accurately to short-term changes in plasma volume since changes in the plasma concentrations of ions would tend to be levelled after some time by the diffusion of the ions to or from other body fluids. In such a case, the expected changes would be of somewhat smaller magnitude than indicated in Fig. 5.

The values obtained for packed cell volume at the start of the experiment were comparable to those obtained by Hess (1963) and Bailey (1964). However, the mean values were lower in the present experiment. This was probably due, in part, to the large number of samples taken over a short period of time.

2) Summary

a) When values were averaged over each treatment, there was no increase in the mean packed cell volume due to cold exposure.

b) There did appear, however, to be a relative increase in packed cell volume due to cold exposure.

D. Eosinophil counts

1) Results and discussion

When the mean concentrations for each treatment were compared, it was found that cold exposure significantly ($P < 0.01$) decreased the mean concentration of circulating eosinophils from 203 to 151/mm^3, but the average rewarm concentration of 164/mm^3 was not significantly different from the average concentration of the cold period (Appendix III, Tables 3 and 5).

The mean eosinophil counts for each sampling time over the three treatment periods are shown in Table 7, Appendix III.

The relative changes in circulating eosinophils with time in the three periods are tabulated in Table 3 and depicted graphically in Fig. 6.

The initial concentration of 220 circulating eosinophils/mm^3 for the cold period was depressed up to 61% by the cold treatment. On the other hand, the rewarm treatment resulted in an increase of up to 110% over that period's initial value of 104 eosinophils/mm^3. Therefore, it is evident that cold causes a large depression of circulating eosinophils.

Due to the results from one sheep which had a low eosinophil count throughout the project, the mean values obtained are about 30% lower than those obtained by Hess (1963).

When comparing Fig. 6 to Fig. 5, it will be noted that the changes in eosinophil count are opposite in direction to the changes that would have to occur if the changes in eosinophil count are to be explained by plasma volume changes indicated on the basis of packed cell volume alterations.

Table 3. Mean[a] per cent changes in eosinophil counts

	Samples	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	94.1	85.8	103.6	90.8	84.4	91.9	88.7	100.3	108.4	112.8	127.4	128.0	111.3
	SE	0.0	22.1	18.0	26.2	19.3	20.9	14.9	16.0	8.6	7.9	18.0	22.0	19.1	20.3
Cold	Actual	100.0	104.8	106.5	94.1	80.6	66.0	66.2	58.1	50.4	48.0	52.6	41.9	38.9	53.0
	SE	0.0	13.8	12.4	10.3	7.0	8.8	6.7	6.8	9.2	5.1	14.2	9.4	8.5	12.3
Rewarm	Actual	100.0	85.9	85.3	118.4	98.6	107.1	127.1	126.1	143.0	199.1	198.0	210.4	169.5	204.6
	SE	0.0	15.9	17.9	24.5	27.2	17.8	22.2	21.7	34.8	37.6	34.4	35.6	31.6	29.2

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 180 cells/mm^3.
First cold sample = 220 cells/mm^3.
First rewarm sample = 104 cells/mm^3.

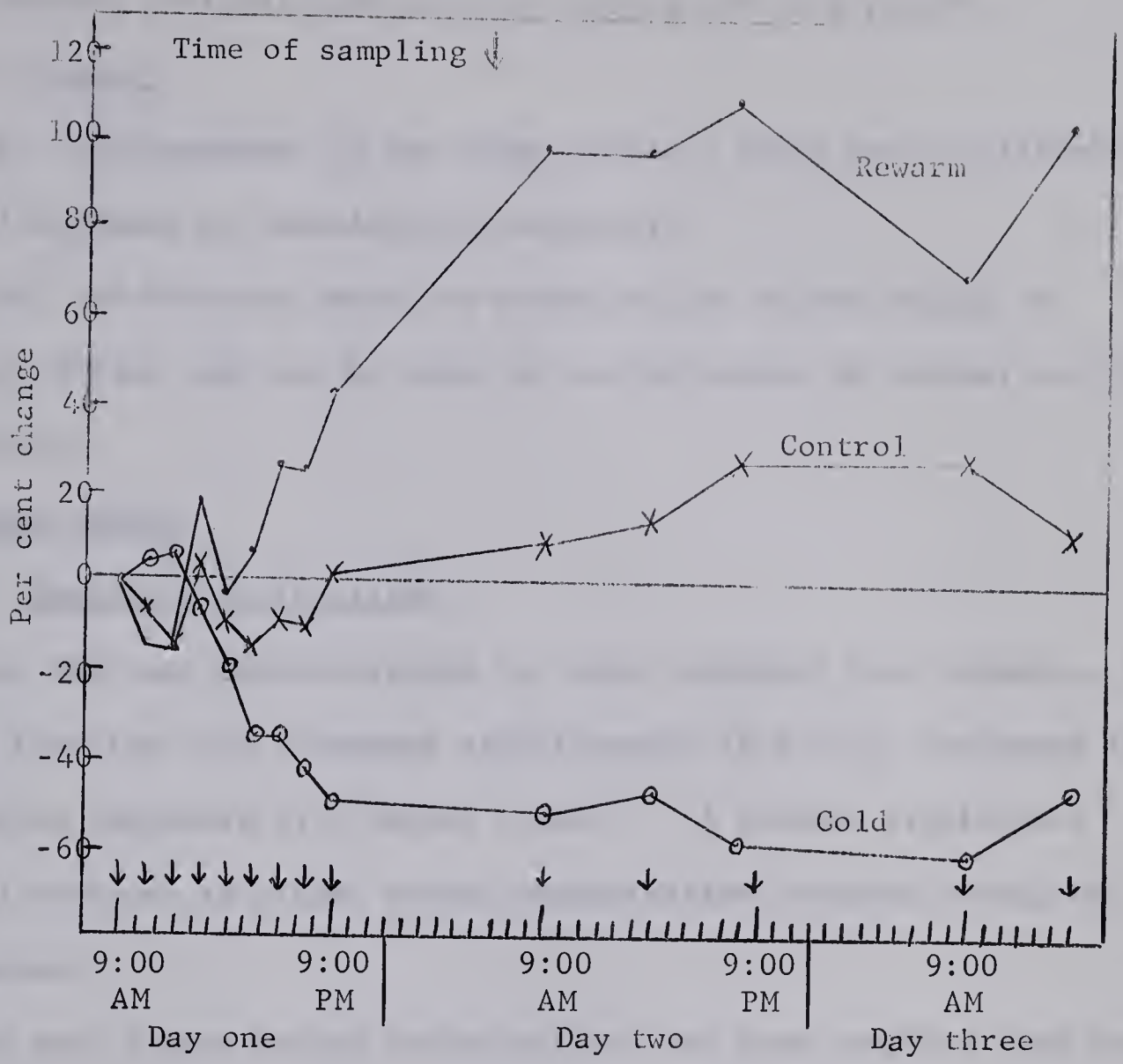


Fig. 6. Effect of temperature on blood eosinophil counts

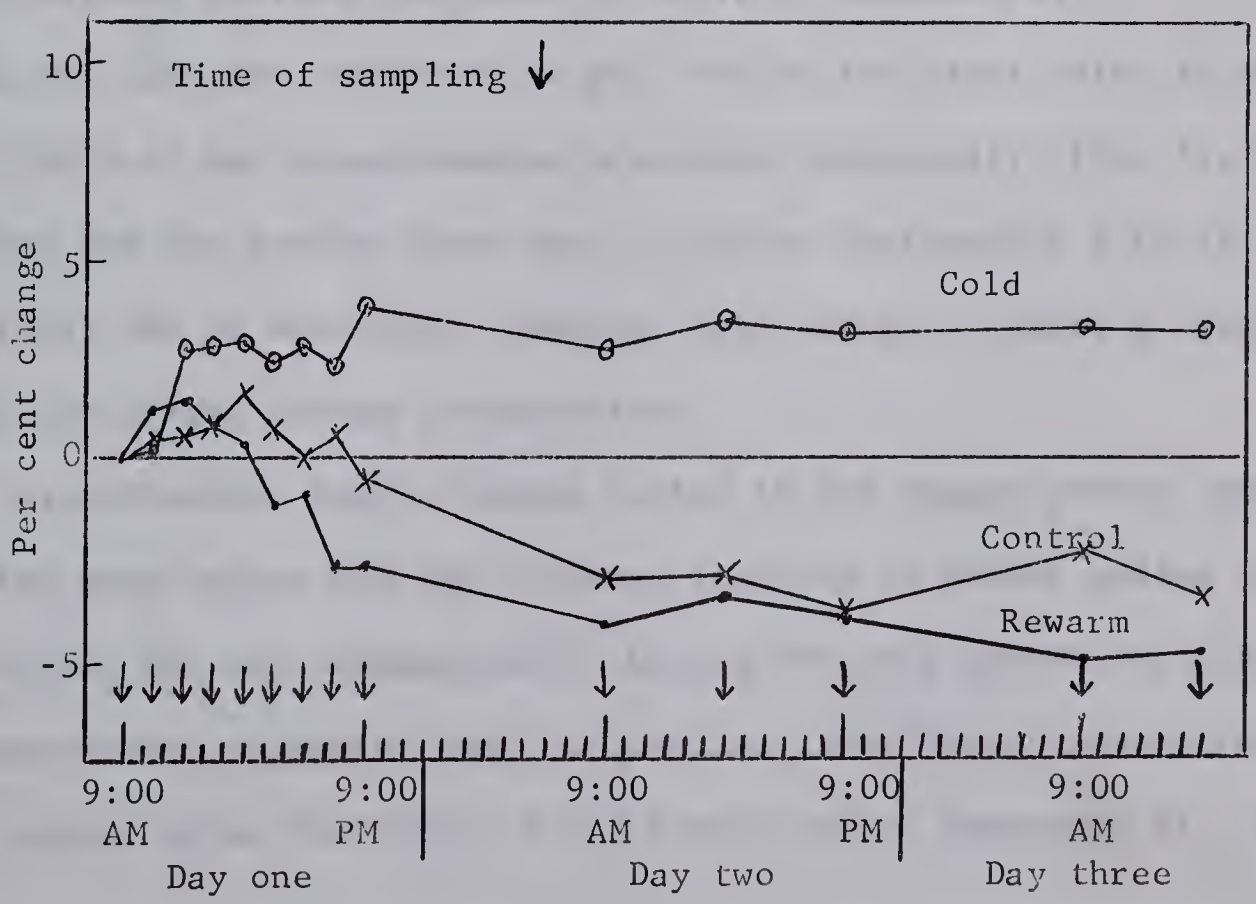
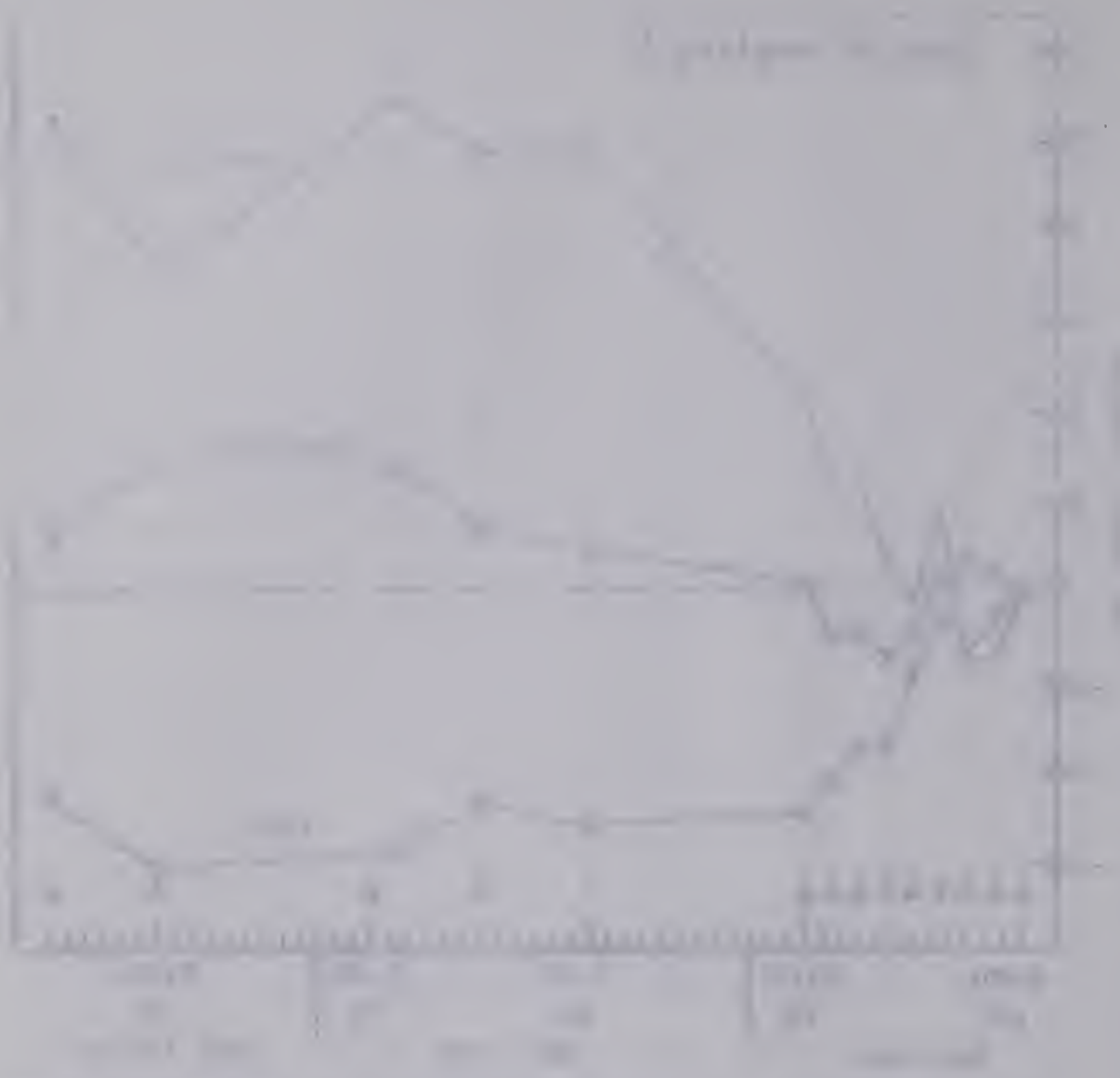


Fig. 7. Effect of temperature on plasma sodium concentration



Therefore, the depression in eosinophil count can be taken to indicate adrenal cortical hyperactivity during the cold period.

2) Summary

a) Cold exposure of the sheep caused a large and significant ($P < 0.01$) decrease in circulating eosinophils.

b) The decrease cannot be accounted for by the change in packed cell volume, and can be taken as an indication of adrenal cortical hyperactivity.

E. Plasma sodium

1) Results and discussion

When the mean concentrations for each treatment were compared, it was found that the cold treatment significantly ($P < 0.01$) increased the plasma sodium (Appendix III, Tables 4 and 5). A further significant ($P < 0.01$) increase in plasma sodium concentration occurred during the rewarm period.

The mean plasma sodium concentrations for each sampling time over the three treatment periods are shown in Table 8, Appendix III.

When the data are converted to per cent of the first value in each treatment (Table 4) and these changes are shown graphically (Fig. 7), the control curve and the rewarm curve show a similar decrease of 3 to 5% after the first day of sampling. However, cold exposure caused a relative increase in the plasma sodium concentration.

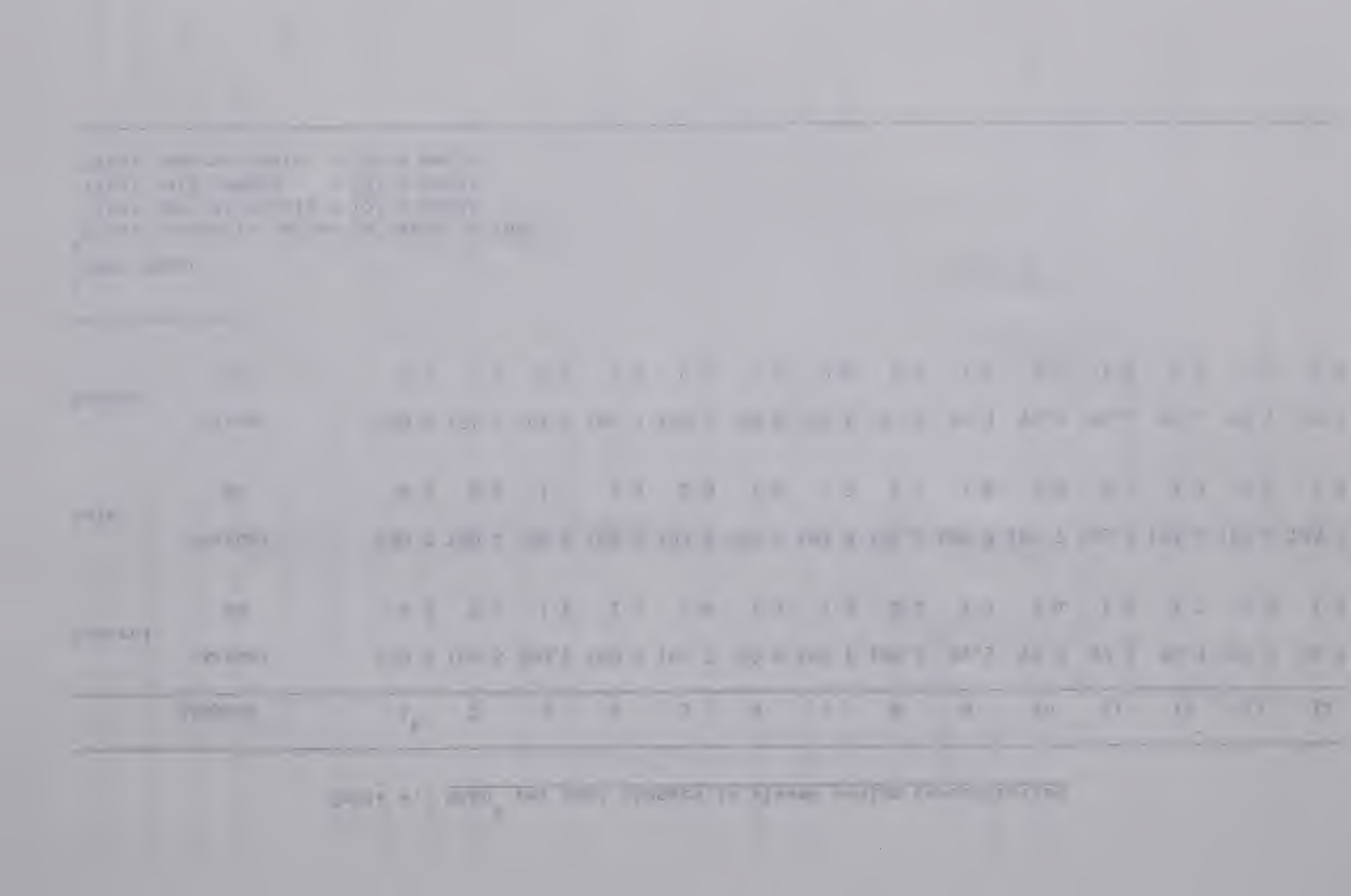
The significantly higher plasma sodium in the rewarm period, when comparing the mean values and the relative lowering of plasma sodium in the same period, are not imcompatible. During the cold period the plasma sodium concentration increased until it reached its greatest concentration which was shortly after the start of the rewarm period (Appendix III, Table 8).

Table 4. Mean[a] per cent changes in plasma sodium concentration

Samples		1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	100.5	100.6	100.9	101.7	100.8	100.1	100.6	99.5	97.0	97.1	96.3	97.8	96.7
	SE	0.0	0.4	1.6	1.7	1.4	1.9	1.5	2.2	2.3	1.4	1.5	1.2	1.0	1.4
Cold	Actual	100.0	100.4	102.8	102.9	103.0	102.5	102.9	102.4	103.9	102.7	103.5	103.2	103.4	103.3
	SE	0.0	0.9	1.7	1.8	2.0	1.8	1.7	1.7	1.8	1.6	0.7	1.5	1.6	1.3
Rewarm	Actual	100.0	101.1	101.9	100.9	100.3	98.8	99.1	97.3	97.3	95.9	96.6	96.1	95.1	95.3
	SE	0.0	1.1	0.6	1.0	0.7	1.4	0.4	1.3	1.6	1.2	1.9	1.8	1.5	1.9

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 151.6 meq/l.
First cold sample = 151.2 meq/l.
First rewarm sample = 159.8 meq/l.



Consequently, the first sample of the rewarm period had a high concentration compared to samples taken during the last 2 days of the rewarm treatment.

When the observed relative changes of plasma sodium in Fig. 7 are compared with the expected changes indicated by changes in packed cell volume shown in Fig. 5, it becomes evident that the majority of the observed changes which occurred in the plasma sodium concentration during cold exposure are in the direction expected on the basis of the change in packed cell volume. The same is true for the control and rewarm changes in plasma sodium concentration except for those occurring during the first 12 hr of these two periods. This agrees with Bailey's (1964) assumption that the change in plasma sodium could be accounted for by the change in packed cell volume.

However, the fact that there was a sustained increase in the plasma sodium concentration during the cold period strengthens the possibility that this increase was due to increased minerocorticoid activity.

The mean values of 150.5, 155.2, and 156.9 meq/l for control, cold, and rewarm treatments, respectively, are very close to the levels found for plasma sodium in sheep by Bailey (1964) and Hess (1963).

2) Summary

a) Cold stress significantly ($P < 0.01$) increased the mean plasma sodium concentration.

b) Rewarming the chamber resulted in a relative decrease of plasma sodium concentration.

c) The direction of these changes can possibly be explained by both changes in plasma volume and in minerocorticoid activity.

F. Plasma potassium

1) Results and discussion

When the mean concentrations for each treatment were compared, it was found that the plasma potassium was significantly ($P < 0.01$) increased in the cold from 4.76 to 4.95 meq/1 (Appendix III, Tables 4 and 5). Rewarming the environment resulted in a significantly lowered mean plasma potassium concentration of 4.52 meq/liter.

The mean plasma potassium concentrations for each sampling time over the three treatment periods are shown in Table 9, Appendix III. Plasma potassium fluctuated greatly in all trials with the most of the increase during cold exposure coming within the first 9 hours.

The relative changes in plasma potassium concentration with time in the three periods are shown in Table 5 and depicted graphically in Fig. 8. In general, the plasma potassium concentration showed a relative increase during the cold treatment and a relative decrease during both the control and rewarm treatments.

When the observed relative changes of plasma potassium in Fig. 8 are compared with the expected changes indicated in Fig. 5, it is seen that the rapid increase in observed plasma potassium during early cold exposure cannot be explained by a change in packed cell volume, although the changes during the two warm periods might be explained partly by this. However, the picture is not very clear because of the fluctuations in the plasma potassium values. These fluctuations possibly explain the diversity of results found in the literature for changes in potassium which are attributed to cold exposure.

The plasma potassium concentrations found are approximately the same as those found by other workers studying sheep (Bailey, 1964; Hess, 1963).

Table 5. Mean[a] per cent changes in plasma potassium concentration

Samples		1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	95.2	96.3	93.6	97.4	94.6	102.3	99.6	93.5	91.6	90.0	92.1	94.2	89.3
	SE	0.0	1.7	6.1	3.6	6.7	4.6	5.9	2.5	6.4	3.1	2.5	2.6	5.7	3.4
Cold	Actual	100.0	100.0	111.0	102.5	104.4	98.7	103.0	97.0	100.1	98.8	103.0	102.2	93.3	96.4
	SE	0.0	4.5	6.0	0.4	4.7	2.6	1.7	4.3	1.6	1.8	5.3	3.2	5.2	0.9
Rewarm	Actual	100.0	100.5	98.8	98.8	100.3	95.5	98.8	94.5	94.2	95.9	93.8	96.9	98.6	101.7
	SE	0.0	4.6	6.7	5.4	5.7	6.2	4.2	4.0	4.8	5.1	6.8	5.5	4.5	5.7

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 5.02 meq/l.
First cold sample = 4.91 meq/l.
First rewarm sample = 4.66 meq/l.

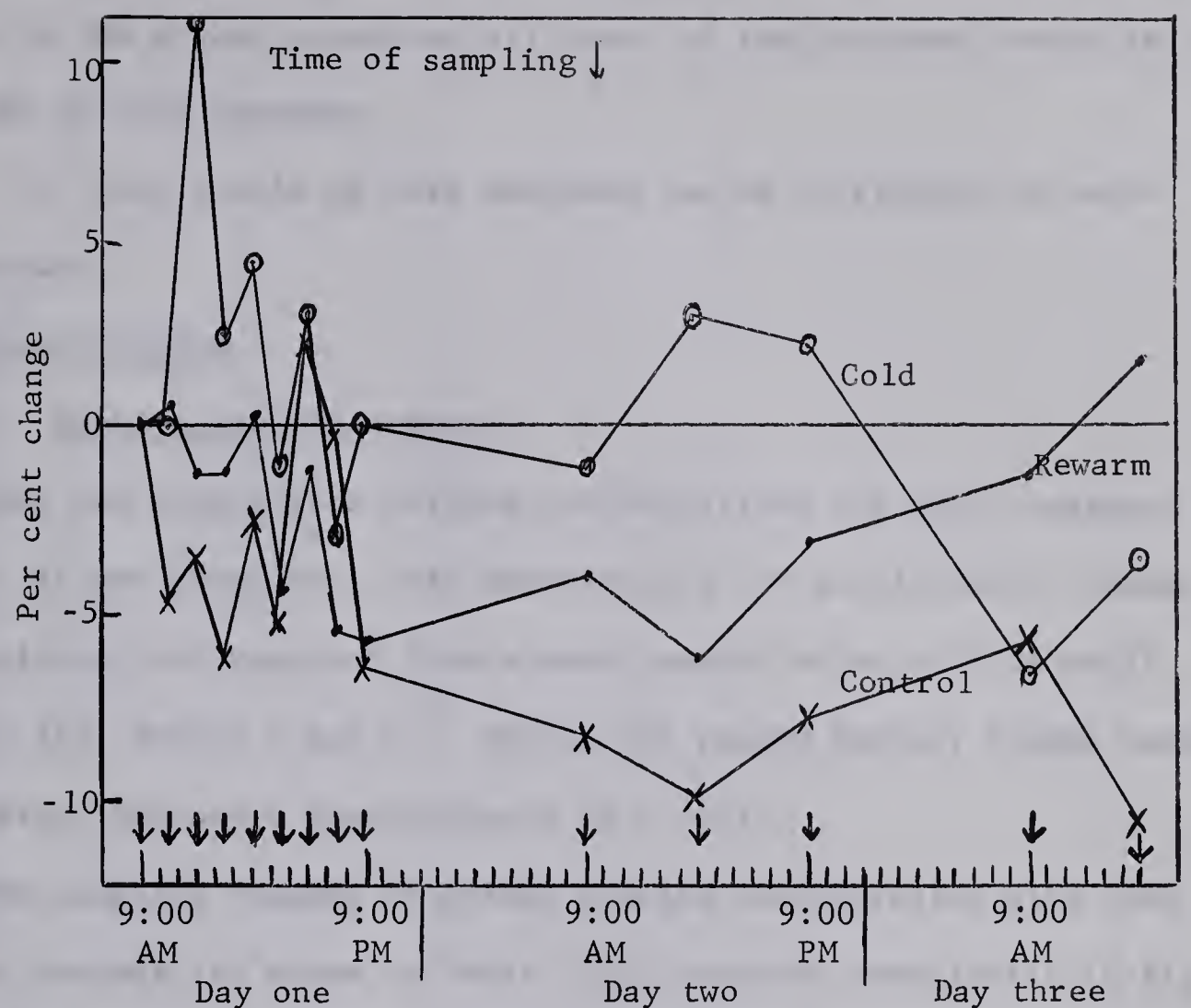


Fig. 8. Effect of temperature on plasma potassium concentration

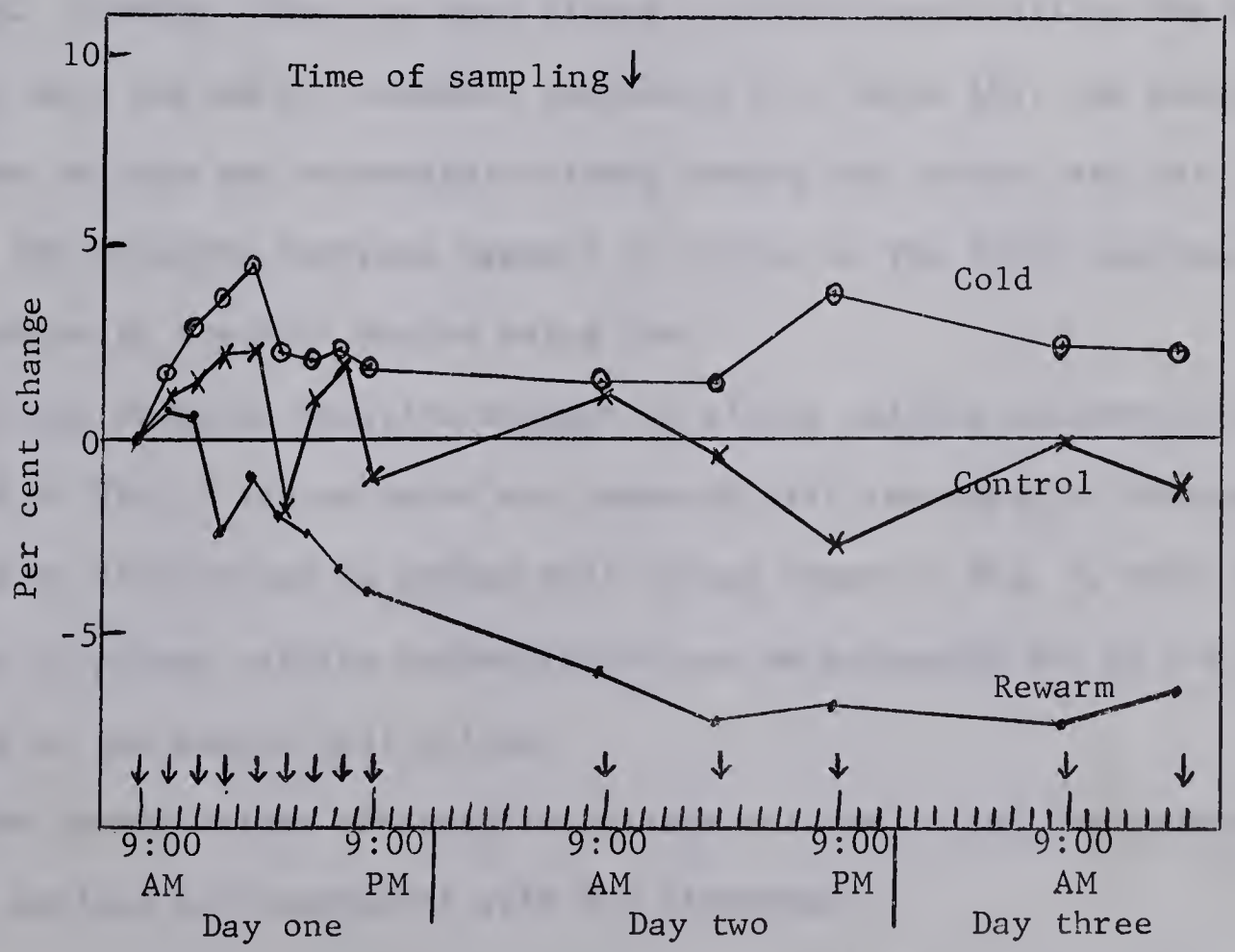
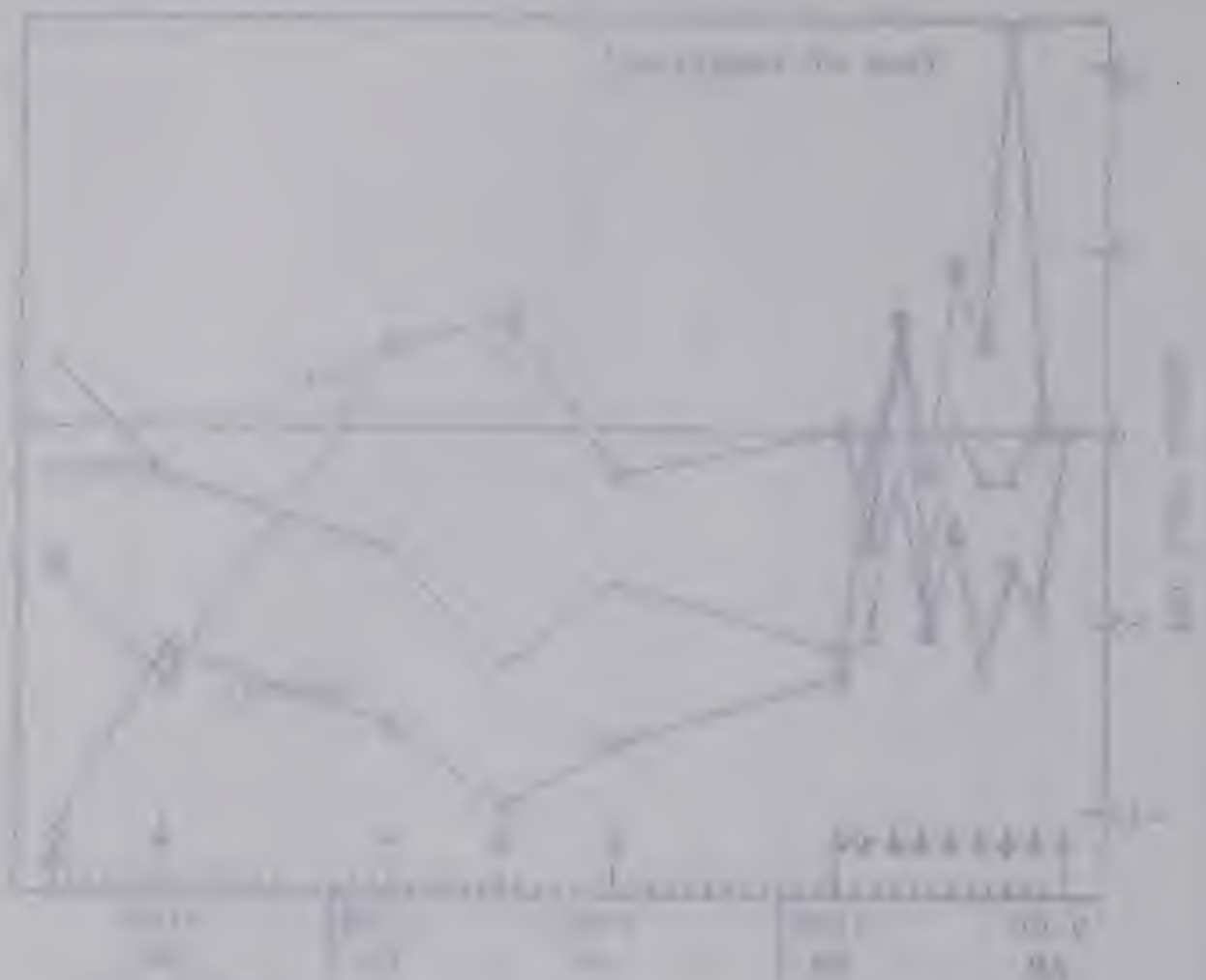


Fig. 9. Effect of temperature on plasma calcium concentration



2) Summary

a) Cold exposure of the sheep caused a significant ($P < 0.01$) increase in the plasma potassium with most of the increase coming in the first 9 hr of cold exposure.

b) Very little of this increase can be attributed to hemo-concentration.

G. Plasma calcium

1) Results and discussion

When the mean plasma calcium concentrations for each treatment were compared, it was found that cold exposure did not significantly change the plasma calcium concentration from a mean control value of 5.01 meq/l (Appendix III, Tables 4 and 5). During the rewarm period, plasma calcium concentration decreased significantly ($P < 0.01$).

The relative changes in plasma calcium concentration with time in the three periods are shown in Table 6 and depicted graphically in Fig. 9. A relative increase in plasma calcium concentration during cold was indicated. However, when the mean plasma calcium concentrations for each sample in each period are examined (Appendix III, Table 10), the results for plasma calcium are exceedingly steady during the control and cold period. The relative increase appears to be due to the first calcium concentration in the cold period being low.

If the observed relative changes of plasma calcium concentration indicated in Fig. 9 are accepted and compared with the expected changes indicated by differences in packed cell volume shown in Fig. 5, most of the variation in plasma calcium concentration can be accounted for by the variation in the packed cell volume.

The steady values obtained for plasma calcium during the control and cold periods are consistent with the literature.

Table 6. Mean[a] per cent changes in plasma calcium concentration

	Samples	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	101.1	101.5	102.1	102.3	98.2	101.1	101.9	99.0	101.2	99.6	97.3	99.9	98.7
	SE	0.0	1.8	2.3	2.2	2.2	4.7	3.4	2.3	1.7	2.0	1.8	1.3	3.0	1.7
Cold	Actual	100.0	101.8	103.0	103.8	104.6	102.4	102.2	102.4	101.9	101.5	101.5	103.8	102.3	102.2
	SE	0.0	1.2	1.7	1.7	1.3	1.4	1.4	1.2	0.9	1.4	0.8	0.6	1.6	1.8
Rewarm	Actual	100.0	101.0	100.7	97.9	99.3	98.2	97.7	96.8	96.2	94.1	92.8	93.1	92.7	93.4
	SE	0.0	0.6	0.7	1.8	1.0	0.7	1.1	1.6	0.9	0.9	1.2	1.5	1.6	1.6

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 4.99 meq/l.
First cold sample = 4.90 meq/l.
First rewarm sample = 5.11 meq/l.

The plasma calcium concentrations obtained were about 0.5 meq/l lower than those obtained for sheep by Bailey (1964). However, his results were similar in that he found no significant difference in the plasma calcium of sheep exposed to the cold.

2) Summary

a) Cold exposure did not significantly affect plasma calcium concentration in the sheep.

b) The decreases in calcium during the rewarm period may be explained by the concurrent decrease in packed cell volume.

H. Plasma magnesium

1) Results and discussion

When the mean concentrations for each treatment were compared, it was found that cold exposure resulted in a significant ($P < 0.01$) decrease in plasma magnesium concentration (Appendix III, Tables 4 and 5). The rewarm treatment resulted in a further significant ($P < 0.01$) decrease in the plasma magnesium. As indicated by the data in Appendix III, Table 11, the decrease during cold exposure was very small.

The relative changes in plasma magnesium concentration with time in the three periods are shown in Table 7 and depicted graphically in Fig. 10. The relative changes are similar to the differences indicated by the plasma means. While the mean concentration for plasma magnesium was lower than for the control during cold exposure, it is interesting to note that during the first day of sampling there was a relative increase in plasma magnesium in the same period. A similar relative increase in plasma magnesium occurred during the control period while a rapid relative decrease occurred over the same time interval in the rewarm period.

The plasma cavities encountered vary along Orbit 1 and 2 from those during Orbits 3 and 4. Despite the general agreement in that the trend is clear, the plasma cavities at Jupiter appear to be smaller.

3) Summary

a) Data required for more definitive answers

Considerations of the theory

b) The decrease in cavities during the Saturn orbits by Pioneer

6. Plasma composition

a) Positive ion density

Even the most conservative estimates of the positive ion density in the plasma cavities (Figure 3b, Orbits 3 and 4) are found that this assumed ionized in a distribution (r^2 3.0) because in plasma composition (Figure 3b, Orbits 3 and 4). The results already resulted in a factor (positive) (r^2 (0.02) because in the plasma composition. It indicated by the data in Figure 3b, Orbit 1) the decrease during this experiment was very small.

The positive charges in plasma composition with time in the three periods are given in Table 3 and depicted graphically in Fig. 10. The positive charges are limited in the measurements obtained by the plasma probe. While the most conservative (or minimum) values have been used for the density calculations, it is interesting to note that during the flyby of Jupiter there was a reliable increase in plasma densities in the ion tails. A similar situation occurs in plasma composition occurred during the normal passage within Jupiter's magnetic tail. Both the inbound and the outbound

Table 7. Mean[a] per cent changes in plasma magnesium concentration

Samples		1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	100.3	103.4	104.9	103.8	103.4	104.2	105.3	100.0	99.6	98.9	95.7	96.1	94.8
	SE	0.0	2.1	3.6	3.2	3.5	1.8	2.8	3.2	2.5	2.2	2.9	3.1	2.1	2.6
Cold	Actual	100.0	100.0	102.2	101.7	100.6	101.9	103.4	99.8	100.6	100.8	100.9	101.3	96.6	97.9
	SE	0.0	1.2	2.1	1.4	0.8	1.2	3.0	1.2	3.0	1.7	3.1	2.9	3.5	0.9
Rewarm	Actual	100.0	97.8	97.1	96.4	94.1	95.6	93.9	93.8	88.7	90.0	91.0	91.5	92.0	91.1
	SE	0.0	1.7	3.2	3.8	3.9	4.5	3.9	4.6	4.2	3.3	4.9	4.9	4.2	4.9

[a] Four sheep.

[b] First sample in series is taken as 100%.
First control sample = 1.79 meq/1.
First cold sample = 1.77 meq/1.
First rewarm sample = 1.80 meq/1.

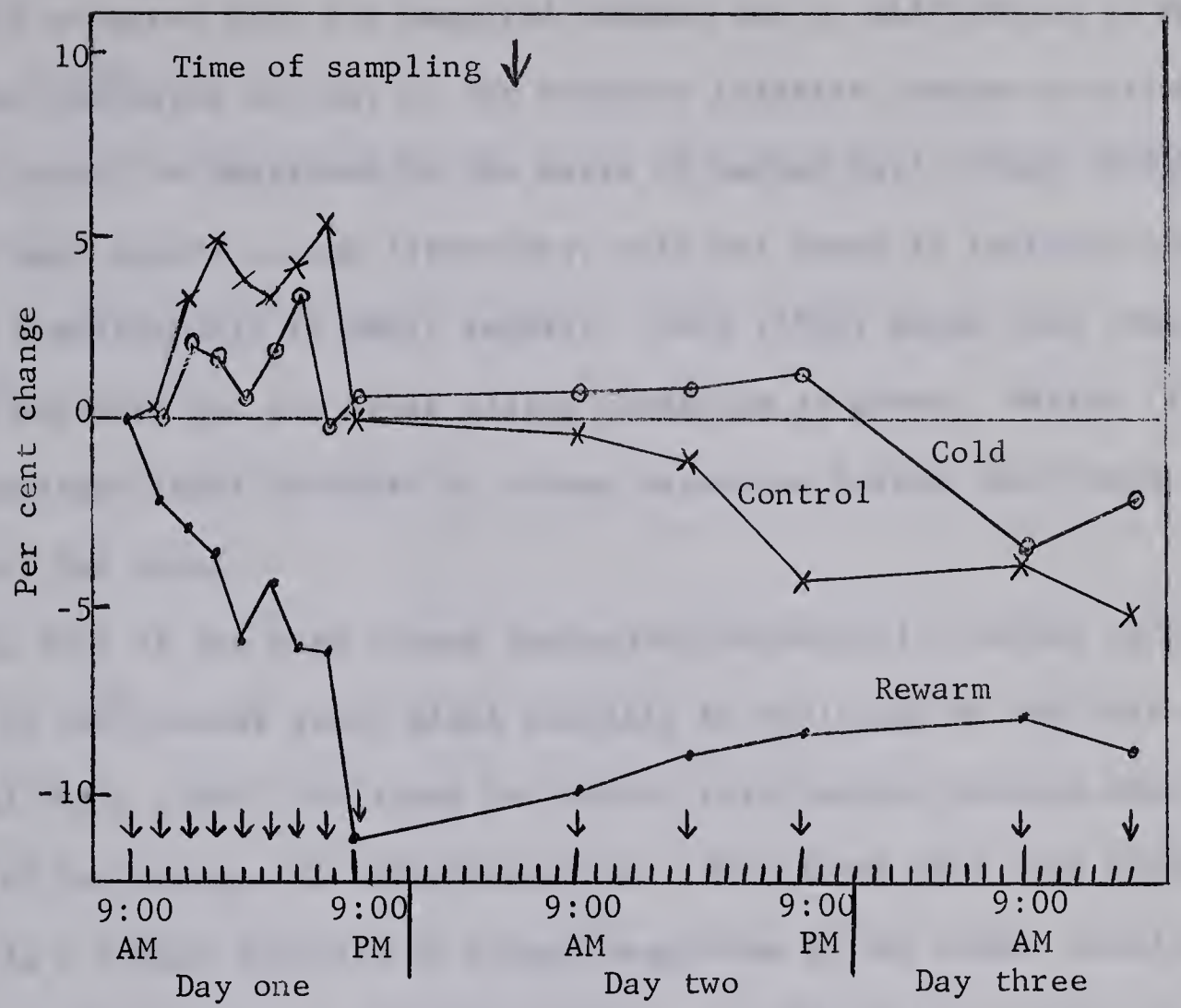


Fig. 10. Effect of temperature on plasma magnesium concentration

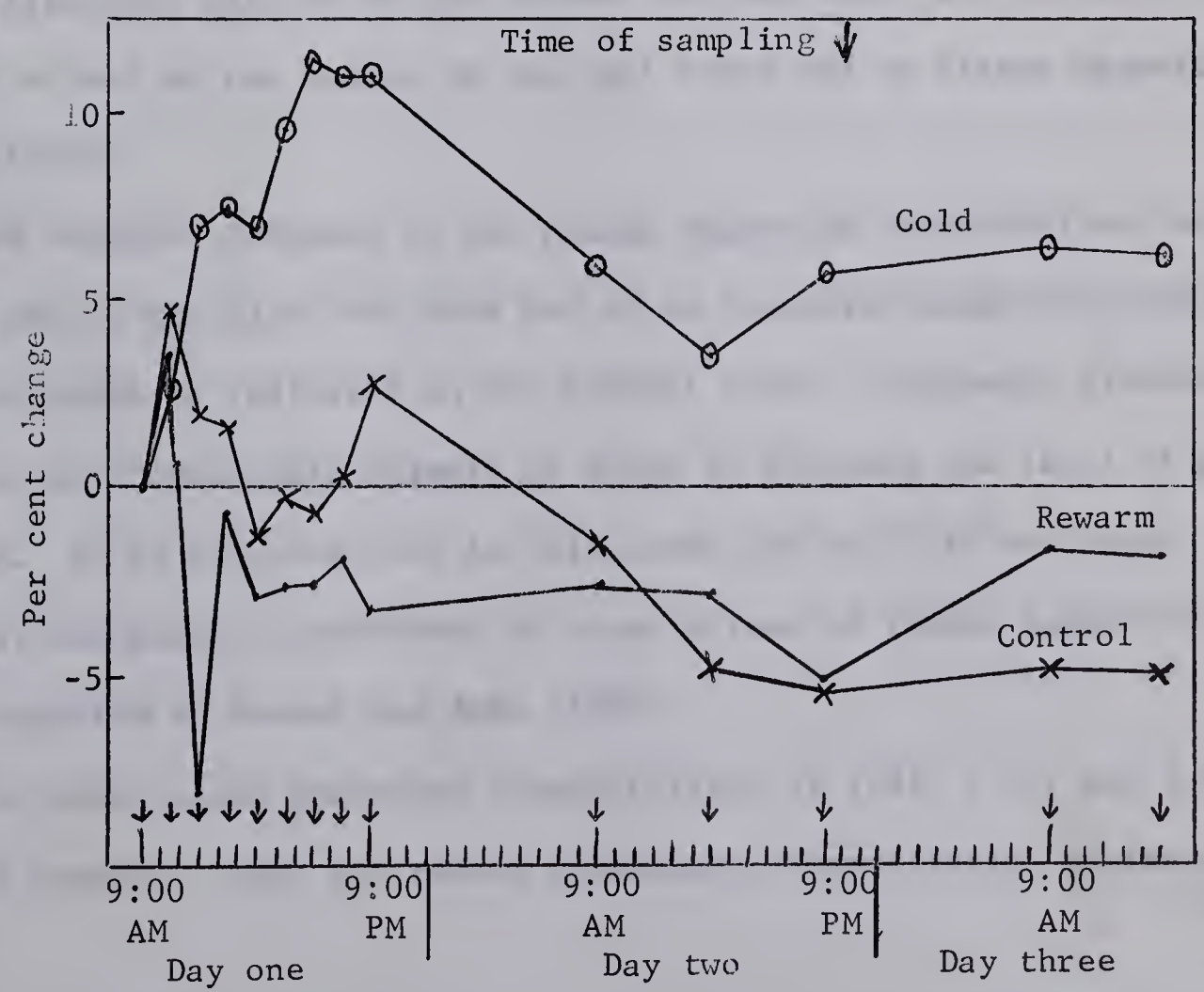
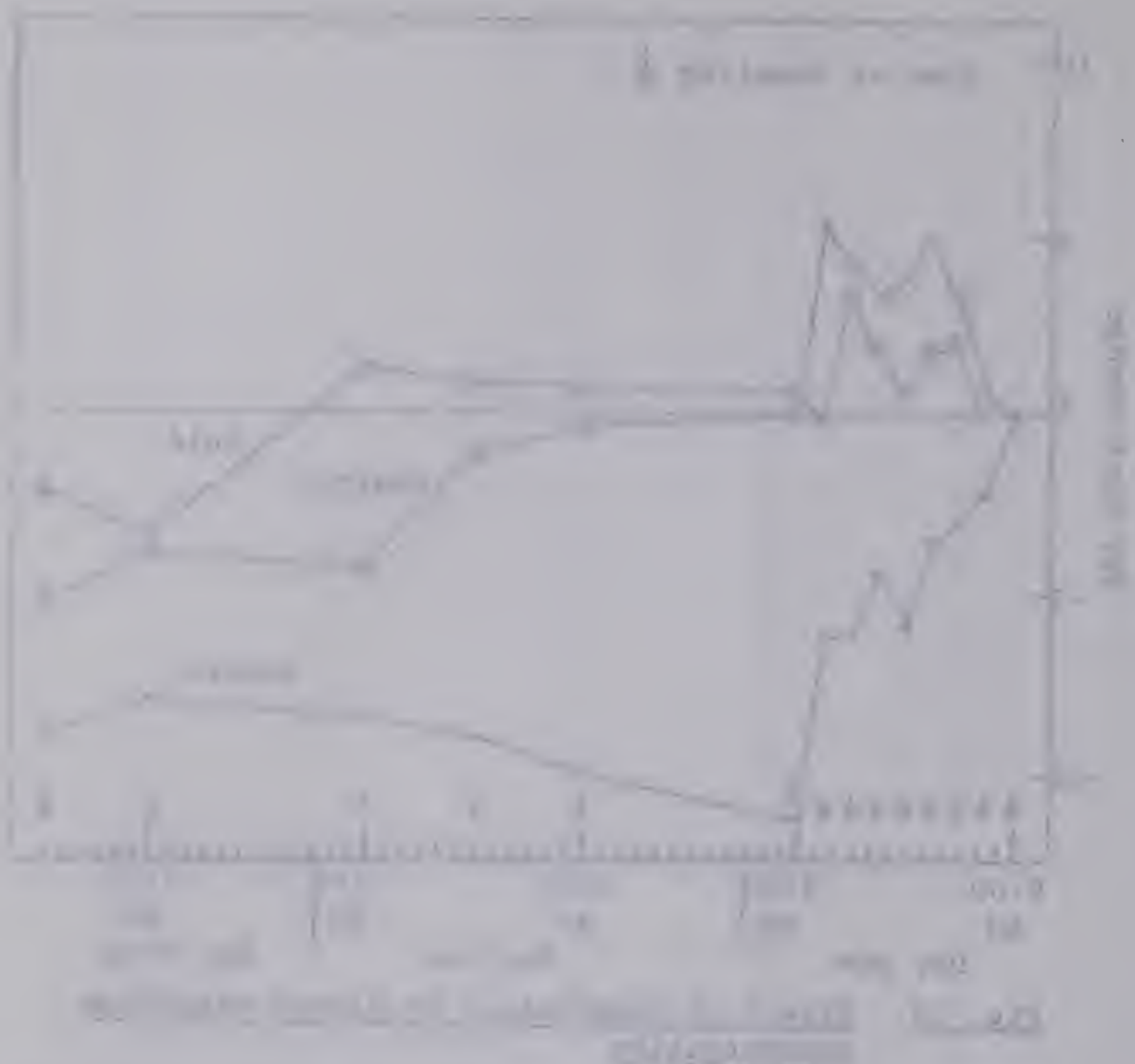
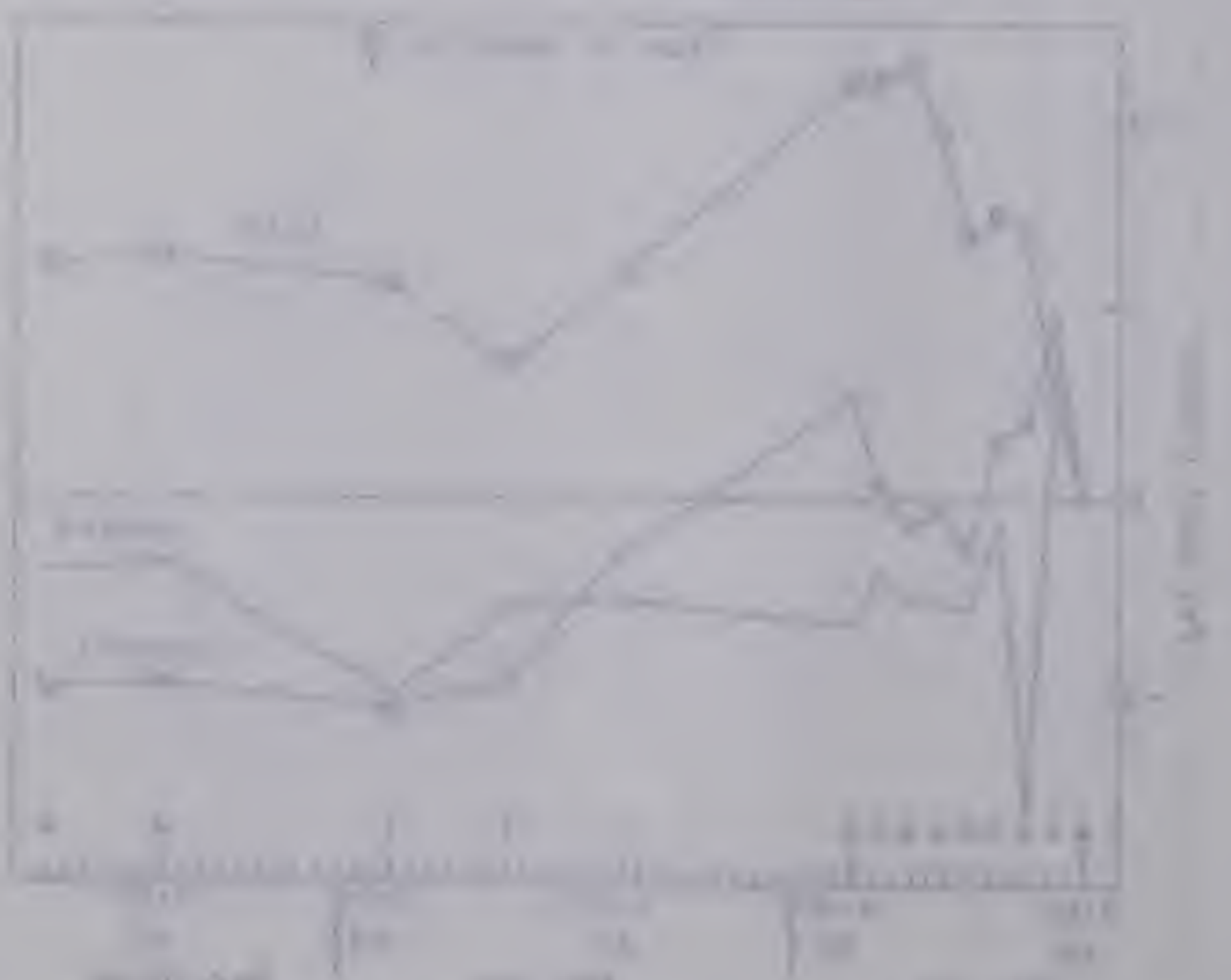


Fig. 11. Effect of temperature on plasma glucose concentration





When the observed relative changes for plasma magnesium shown in Fig. 10 are compared with the expected changes due to differences in packed cell volume indicated in Fig. 5, the observed relative changes in plasma magnesium cannot be explained on the basis of packed cell volume changes.

In most papers in the literature, cold was found to increase plasma magnesium significantly in small animals. Hess (1963) found that long-term cold exposure did not alter plasma magnesium in sheep. Bailey (1964) found a nonsignificant increase in plasma magnesium during short-term cold exposure of the sheep.

The fall of the mean plasma magnesium concentration during cold exposure in the present study might possibly be explained by the work of Nowell and White (1963) who found an inverse relationship between daily photoperiod and changes in serum magnesium. They found that cold which resulted in a slight decrease in plasma magnesium in the summer resulted in a significant increase of plasma magnesium in the winter. In the present study, a light was left on in the chamber day and night and may have had a similar effect as the number of daylight hours had on plasma magnesium concentrations.

The observed decrease in the plasma magnesium concentration during the cold period may also have been due to an increased minerocorticoid production which is indicated in the present study. Increased aldosterone production in normothermic animals is known to decrease the level of plasma magnesium. It is possible that in this study the cold did not cause sufficient peripheral hypothermia to cause a loss of tissue magnesium such as that reported by Moussa and Boba (1960).

The mean plasma magnesium concentrations of 1.81, 1.77, and 1.68 meq/l for control, cold, and rewarm treatments, respectively, in the present

experiment are midway between the results obtained by Bailey (1964) and Hess (1963).

2) Summary

a) Cold exposure decreased the plasma magnesium concentration in the sheep.

b) Rewarming further decreased plasma magnesium.

J. Plasma glucose

1) Results and discussion

When the mean concentrations for each treatment were compared, it was found that cold exposure resulted in a significant ($P < 0.01$) increase from 64.4 to 67.1 mg/100 ml in the plasma glucose concentration (Appendix III, Tables 3 and 5). The rewarm period resulted in a significant ($P < 0.01$) decrease from the cold mean value to an average of 63.8 mg/100 milliliters.

The average concentrations for each sample time indicate that the increase in plasma glucose concentration in cold-exposed sheep is greater than that shown by the mean values for the treatment periods (Appendix III, Table 12).

The relative data shown in Table 8 and depicted graphically in Fig. 11 demonstrate that plasma glucose levels rapidly increased due to exposure to cold. The raised plasma glucose concentrations were highest during the first 12 hr and showed an increase of about 5% over the first value throughout the remainder of the cold period.

When the observed relative changes in plasma glucose shown in Fig. 11 are compared with the expected changes indicated by differences in packed cell volume in Fig. 5, it can be seen that the observed differences in plasma glucose cannot be entirely attributed to variations in the packed cell volume.

Table 8. Mean[a] per cent changes in plasma glucose concentration

Samples		1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Actual	100.0	104.8	102.0	101.7	98.8	99.8	99.4	100.3	102.7	98.5	95.2	94.6	95.2	95.1
	SE	0.0	4.1	1.4	2.6	2.0	3.4	2.1	3.3	2.6	3.2	1.9	4.9	2.1	2.1
Cold	Actual	100.0	102.7	106.9	107.5	106.9	109.5	111.4	111.0	111.0	105.9	103.6	105.8	106.4	106.2
	SE	0.0	1.4	2.5	3.6	3.4	3.8	3.8	4.6	4.3	3.4	1.1	6.6	2.5	2.7
Rewarm	Actual	100.0	103.4	91.9	99.4	97.2	97.4	97.5	98.2	96.8	97.5	97.3	94.8	98.5	98.4
	SE	0.0	2.7	12.4	2.1	5.0	1.0	2.8	0.7	1.4	1.4	1.7	2.5	2.8	4.1

[a]Four sheep.

[b]First sample in series is taken as 100%.
First control sample = 65.2 mg/100 ml.
First cold sample = 62.9 mg/100 ml.
First rewarm sample = 65.3 mg/100 ml.

| | | |

The values obtained for plasma glucose are within the range for sheep expressed by Dukes (1955) and are similar to the concentrations found in sheep by Reid (1962). Reid usually found a larger increase in plasma glucose due to cold exposure.

2) Summary

a) Cold exposure significantly ($P < 0.01$) increased plasma glucose in the sheep.

b) The relative increase in plasma glucose was greatest during the first 12 hr of cold exposure.

c) Not all of the increase could be accounted for by a concurrent increased packed cell volume.

GENERAL DISCUSSION

The marked reduction in circulating eosinophils during cold stress shown in Fig. 6 indicates an early adrenal cortical hyperactivity due to cold similar to that proposed by Boulouard (1963) and many others. The increase in plasma sodium concentration (Fig. 7) during the time that the adrenal cortex was becoming more active suggests a concurrent minerocorticoid activity as proposed by Munday and Blane (1961). While it was not determined whether the minerocorticoid was aldosterone or deoxycorticosterone, it is reasonable to assume that it was aldosterone. Aldosterone is the most highly active minerocorticoid and is the predominant one secreted by the adrenal cortex of the sheep. Aldosterone is known to cause sodium retention, and an increased secretion of aldosterone in the cold would account for the sustained increase in the plasma sodium concentration observed during cold exposure in the present study.

The elevation in the plasma glucose concentration during cold exposure also indicates adrenal gland hyperactivity, probably involving both the secretion of epinephrine by the adrenal medulla and nerve endings and glucocorticoid secretion by the adrenal cortex. In comparing the results of the present study with those of Reid (1962) it is probable that the elevated plasma glucose concentration during the first 12 hr of cold exposure was due to increased glycogenolysis caused by the action of epinephrine. The drop that occurred over the next 12 hr may represent a subsequent decrease in glycogenolysis. The final levelling off of the plasma glucose concentration in the cold at a value which was still greater than the control value could then be explained by gluconeogenesis mediated by glucocorticoids.

The higher relative plasma concentrations of potassium (Fig. 8), calcium (Fig. 9), and magnesium (Fig. 10) during the first 12 hr of cold exposure correspond to a lowered jugular vein temperature over the same 12-hr period. The change in jugular vein temperature indicates a temporary hypothermia which would result in elevated concentrations of these three ions in the plasma.

The packed cell volume was increased during the cold exposure indicating hemoconcentration. The packed cell volume was used as an indicator of plasma volume but the use of this parameter in the interpretation of longer term changes in plasma solute concentrations may be limited in the present study because the constancy of the total volume of circulating erythrocytes was not determined.

Whereas the packed cell volume is probably a good indicator of short-term changes in plasma volume, the longer term changes in ion concentrations which were observed are probably real changes involving both the plasma and the extracellular fluid. If these changes were not real, the plasma ion concentrations would tend to be compensated by diffusion to or from the extracellular fluid.

When comparing the values for the parameters from the preliminary experiment shown in Fig. 1 to the values of all the later figures, it becomes apparent that different stressors were at work in the preliminary trial. The changes in the preliminary trial returned to normal after about 12 hours. This is similar to the short-term effects of emotional stress on cortisol found by Reid and Mills (1962).

On the other hand, during the later experiment the changes were prolonged throughout the 54 hr of cold. This is similar to the effects of cold, wind, and rain on plasma cortisol and glucose found by Reid (1962).

During the experiment there were signs of slight emotional stress during the early parts of the period. An initial stress caused by sampling is apparent during the control period in particular. A decrease in eosinophil numbers occurred during the first 12 hr of the control period. At the same time, a slight increase occurred in plasma sodium (Fig. 7), plasma magnesium (Fig. 10), and plasma glucose (Fig. 11).

In the interpretation of the present data, comparisons between treatment means are instructive in some instances but do not accurately indicate the treatment effects in others. As the majority of the samples were taken during the first day of each treatment, these values strongly influenced the treatment mean. In some of the parameters the greatest change did not occur until after the first day and hence did not greatly affect the mean treatment value. The treatment means therefore have served, in some cases, as guides for interpretation, whereas primary emphasis has been placed on changes in the different parameters with respect to time, taking into consideration the standard errors of individual sample means.

GENERAL SUMMARY

1) Both the jugular vein and rectal temperatures were lowered by cold exposure. The mean jugular vein temperatures were 0.4 to 0.5 C lower than the mean rectal temperatures. The rectal temperatures showed a thermal lag when compared to the jugular vein temperatures.

2) Cold exposure resulted in a relative increase in packed cell volume.

3) Cold exposure resulted in a large decrease of circulating eosinophils indicating adrenal cortical hyperactivity. The eosinopenia persisted throughout the cold period.

4) As cold exposure decreased the circulating eosinophils, the concentration of plasma sodium increased indicating increased minerocorticoid activity,

5) The plasma concentrations of potassium and magnesium increased during the first 12 hr of cold exposure. A similar relative increase in calcium may have been due to a low initial calcium value in the cold period. A depression of jugular vein temperature which occurred in this same interval indicated a temporary hypothermia which would lead to the observed increases in plasma, potassium, and magnesium.

6) The plasma glucose concentration was elevated by the cold exposure. The high concentrations seen during the first 12 hr of cold decreased subsequently to a steady level which was still greater than the control value. These changes in the plasma glucose also suggested adrenal hyperactivity due to exposure to cold.

4) Only the Jupiter file was in its regular drawer. Leaving no
note behind. The other Jupiter notes [illegible] Dec 31 to Jan 2
have [illegible] the data readily [illegible]. The second [illegible] missed
a shower log [illegible] in the Jupiter data [illegible].

5) Cold [illegible] [illegible] is a [illegible] [illegible] in [illegible] cell
[illegible].

6) [illegible] [illegible] is a [illegible] [illegible] [illegible] [illegible]
[illegible] [illegible] [illegible]. The [illegible] [illegible]
[illegible] the cold [illegible].

7) As cold [illegible] [illegible] the [illegible] [illegible], the
[illegible] of plasma [illegible] [illegible] [illegible] [illegible]
cold [illegible].

8) The plasma [illegible] [illegible] [illegible] [illegible]
[illegible] the fiber it is of cold [illegible]. A plasma [illegible] [illegible]
[illegible] may have [illegible] a low [illegible] [illegible] [illegible]
a depression of Jupiter. Data [illegible] [illegible] [illegible] [illegible]
[illegible] a [illegible] [illegible] [illegible] [illegible] [illegible]
in plasma, pressure, and magnetic.

9) The plasma [illegible] [illegible] [illegible] by the cold [illegible]
The ship [illegible] [illegible] during the [illegible] it is of cold [illegible]
[illegible] in a [illegible] [illegible] [illegible] [illegible] [illegible]
[illegible] changes in the plasma [illegible] [illegible] [illegible]
due to [illegible] in cold.

7) The stressor agent in the preliminary trial, where the sheep were moved between periods and the temperature was not lowered as much or effectively, was emotional. However, the stressor agent during the later experiment was cold and the sheep did not show signs of becoming acclimatized after 54 hr of cold exposure.

BIBLIOGRAPHY

Adams, T. 1963. Body-temperature regulation in the normal and cold-acclimatized cat. J. Appl. Physiol. 18:772-777.

Adolf, E. F., and G. W. Molnar. 1946. Exchanges of heat and tolerances to cold in men exposed to outdoor weather. Amer. J. Physiol. 146:507-537.

Aikawa, J. K., D. R. Harms, and J. Z. Reardon. 1960. Effect of cortisone on magnesium metabolism in the rabbit. Amer. J. Physiol. 199:229-230.

Andersson, B., A. H. Brook, C. C. Gale, and B. Hökfelt. 1964a. The effect of a ganglionic blocking agent on the thermoregulatory response to preoptic cooling. Acta Physiol. Scand. 61:393-399.

Andersson, B., L. Ekman, C. C. Gale, and J. W. Sunsten. 1963. Control of thyrotrophic hormone (TSH) secretion by the "heat loss center". Acta Physiol. Scand. 59:12-33.

Andersson, B., C. C. Gale, B. Hökfelt, and A. Ohga. 1964b. Relation of preoptic temperature to the function of the sympathico-adrenomedullary system and the adrenal cortex. Acta Physiol. Scand. 61:182-191.

Andersson, B., R. Grant, and B. Larsson. 1956. Central control of heat loss mechanisms in the goat. Acta Physiol. Scand. 37:261-280.

Bailey, C. B. 1964. Effect of environmental temperature on feed digestion, water metabolism, body temperature, and certain blood characteristics of sheep. Can. J. Animal Sci. 44:68-75.

Baker, D. G. 1960a. Electrolyte metabolism in the rat exposed to a low environmental temperature. II. Can. J. Biochem. Physiol. 38:205-211.

Baker, D. G. 1960b. Influence of cold exposure on electrolyte metabolism. Fed. Proc. 19 (Suppl. 5):125-130.

Baker, D. G., and E. A. Sellers. 1957. Electrolyte metabolism in the rat exposed to low environmental temperature. Can. J. Biochem. Physiol. 35:631-636.

Barbour, H. G., E. A. McKay, and W. P. Griffith. 1943. Water shifts in deep hypothermia. Amer. J. Physiol. 140:9-19.

Bass, D. E., D. C. Fainer, R. K. Blaisdell, and F. Daniels, Jr. 1951. Adrenal cortical activity and hematological changes in man during cold acclimation. Fed. Proc. 10:10. (Abstr.)

Bass, D. E., and E. Henschel. 1956. Responses of body fluid compartments to heat and cold. Physiol. Rev. 36:128-144.

Beaton, J. R. 1961. Further observations on metabolic alterations in the hypothermic rat. Can. J. Biochem. Physiol. 39:1-8.

Benzinger, T. H. 1961. The quantitative mechanism and the sensory receptor organ of human temperature control in warm environment. Ann. Internal Med. 54:685-699.

Benzinger, T. H. 1963. Peripheral cold- and central warm-reception, main origins of human thermal discomfort. Proc. Nat. Acad. Sci. 49:832-839.

Binnion, P. F., J. O. Davis, T. C. Brown, and M. J. Olichney. 1965. Mechanisms regulating aldosterone secretion during sodium depletion. Amer. J. Physiol. 208:655-661.

Blaxter, K. L. 1962. The effect of environment on energy metabolism, p. 116-148. In K. L. Blaxter, The energy metabolism of ruminants. Charles C. Thomas, Springfield, Ill.

Bligh, J. 1957. The relationship between the temperature in the rectum and of the blood in the bicarotid trunk of the calf during exposure to heat stress. J. Physiol. 136:393-403.

Booker, W. M. 1960. Relation of ascorbic acid to adrenocortical function during cold stress. Fed. Proc. 19 (Suppl. 5): 94-96.

Boulouard, R. 1963. Effects of cold and starvation on adrenocortical activity of rats. Fed. Proc. 22:750-754.

Boulouard, R., and R. Buzalkov. 1963. Influence de la mise en hypothermie et du réchauffement sur le taux des hormones cortico-surrénaliennes du plasma chez le rat (in French, English Summary). Ann. Endocrinol. (Paris) 24:157-167.

Care, A. D., and D. B. Ross. 1963. The role of the adrenal cortex in magnesium homeostasis and in the aetiology of hypomagnesaemia. Res. Vet. Sci. 4:24-38.

Carlson, L. D. 1960. Nonshivering thermogenesis and its endocrine control. Fed. Proc. 19 (Suppl. 5):25-30.

Carlson, L. D. 1962. Temperature. Ann. Rev. Physiol. 24:85-108.

Carlson, L. D. 1963. Temperature regulation and cold acclimation. Physiologist 6:29-39.

Carr, M. H., and H. A. Frank. 1956. Improved method for determination of calcium and magnesium in biologic fluids by EDTA titration. Amer. J. Clin. Path. 26:1157-1168.

Chaffee, R. R. J., W. W. Mayhew, M. Drebin, and Y. Cassuta. 1963. Studies on thermogenesis in cold-acclimated birds. Can. J. Biochem. Physiol. 41:2215-2220.

Chang, C. B., and W. C. Shoemaker. 1963. Effect of hypothermia on red cell volumes. J. Thoracic Cardiovas. Surg. 46:117-124.

Chowers, I., H. T. Hammel, S. B. Stromme, and S. M. McCann. 1964. Comparison of effect of environmental and preoptic cooling on plasma cortisol levels. Amer. J. Physiol. 207:577-582.

Cope, C. L., and J. Pearson. 1963. Aldosterone secretion in magnesium deficiency. Brit. Med. J. 2:1385-1386.

Crawford, A. L., M. J. Henderson, D. R. Hawkins, and R. E. Haist. 1965. The effect of glucagon on blood sugar and inorganic phosphorous levels in normothermic and hypothermic rats. Can. J. Physiol. Pharmacol. 43:601-610.

D'Amato, H. E. 1954. Thiocyanate space and the distribution of water in the musculature of the hypothermic dog. Amer. J. Physiol. 178: 143-147.

Davis, T. R. A. 1963. Nonshivering thermogenesis. Fed. Proc. 22:777-782.

Deb, C., and J. Hart. 1956. Hematological and body fluid adjustments during acclimation to a cold environment. Can. J. Biochem. Physiol. 34:959-966.

Denton, D. A. 1964. Angiotensin, electrolytes and aldosterone. Australasian Ann. Med. 13:121-135.

Denton, D. A., J. R. Goding, and R. D. Wright. 1960. The control of aldosterone secretion, p. 373-391. In E. B. Astwood, Clinical endocrinology. Grune and Stratton, New York, N.Y.

Dukes, H. H. 1955. Temperature regulation, p. 636-653. In H. H. Dukes, The physiology of domestic animals. 7th ed., Comstock, New York, N.Y.

Eliot, J. W., R. A. Bader, and D. E. Bass. 1949. Blood changes associated with cold diuresis. Fed. Proc. 8:41. (Abstr.)

Elliot, H. W., and J. M. Crismon. 1947. Increased sensitivity of hypothermic rats to injected potassium and the influence of calcium, digitalis and glucose on survival. Amer. J. Physiol. 151:366-372.

Evans, C. L. 1956. The temperature and heat-balance of the body, p. 1056-1078. In C. L. Evans, Principles of human physiology. 12th ed., J.& A. Churchill Ltd., London.

Everett, N. B., and L. Matson. 1961. Red cell and plasma volumes of the rat and of tissues during cold acclimation. J. Appl. Physiol. 16: 557-561.

Fuhrman, F. A., and J. M. Crismon. 1947. The influence of acute hypothermia on the rate of oxygen consumption and glycogen content of the liver and on blood glucose. Amer. J. Physiol. 149:552-560.

Fuhrman, G. J., and F. A. Fuhrman. 1963. Utilization of glucose by the hypothermic rat. Amer. J. Physiol. 205:181-183.

Fuhrman, G. J., and F. A. Fuhrman. 1964. Effect of temperature on metabolism of glucose in vitro. Amer. J. Physiol. 207:849-852.

Fusco, M. M., J. D. Hardy, and H. T. Hammel. 1961. Interaction of central and peripheral factors in physiological temperature regulation. Amer. J. Physiol. 200:572-580.

Hammel, H. T., D. C. Jackson, J. A. J. Stolwijk, J. D. Hardy, and S. R. Stromme. 1963. Temperature regulation by hypothalamic proportional control with an adjustable set point. J. Appl. Physiol. 18:1146-1154.

Han, P. W., and J. R. Brobeck. 1961. Deficits of temperature regulation in rats with hypothalamic lesions. Amer. J. Physiol. 200:707-710.

Hanna, S., and I. MacIntyre, 1960. The influence of aldosterone on magnesium metabolism. Lancet 2:348-350.

Hannon, J. P. 1960. Intermediary glucose metabolism in the cold-acclimatized rat. Fed. Proc. 19 (Suppl. 5):100-105.

Hannon, J. P., E. Evonuk, and A. M. Larson. 1963. Some physiological and biochemical effects of norepinephrine in the cold-acclimatized rat. Fed. Proc. 22:783-788.

Hannon, J. P., A. M. Larson, and D. W. Young. 1958. Effect of cold acclimatization on plasma electrolyte levels. J. Appl. Physiol. 13: 239-240.

Hart, J. S. 1958. Metabolic alterations during chronic exposure to cold. Fed. Proc. 17:1045-1054.

Heagy, F. C., and A. C. Burton. 1947. The effect of magnesium on body temperature in the dog. Fed. Proc. 6:126. (Abstr.)

Henneman, D. H., J. P. Bunker, and W. R. Brewster, Jr. 1958. Immediate metabolic response to hypothermia in man. J. Appl. Physiol. 12: 164-168.

Hercus, V. M., and S. Bowman. 1959. Cation studies during experimental hypothermia. Australasian Ann. Med. 8:123-128.

Héroux, O. 1960. Adjustments of the adrenal cortex and thyroid during cold acclimation. Fed. Proc. 19 (Suppl. 5):82-85.

Héroux, O., and J. S. Hart. 1954. Cold acclimation and adrenal cortical activity as measured by eosinophil levels. Amer. J. Physiol. 178: 453-456.

Hess, E. A. 1963. Effect of low environmental temperatures on certain physiological responses of sheep. Can. J. Animal Sci. 43:39-46.

Hodgman, C. D., R. C. Weast, and S. M. Selby. 1960. Relative humidity from wet and dry bulb thermometers (Cent. scale), p. 2496-2497. In C. D. Hodgman, R. C. Weast, and S. M. Selby, Handbook of chemistry and physics. 42nd ed., The Chemical Rubber Publishing Co., Cleveland, Ohio.

Hoijer, D. J. 1960. Metabolic function of ascorbic acid in acclimation to cold. Fed. Proc. 19 (Suppl. 5):90-93.

Horton, R., and E. G. Biglieri. 1962. Effect of aldosterone on the metabolism of magnesium. J. Clin. Endocrinol. Metab. 22:1187-1192.

Horvath, S. M., A. Rubin, and E. L. Foltz. 1950. Thermal gradients in the vascular system. Amer. J. Physiol. 161:316-322.

Hume, D. M., and R. H. Egdahl. 1959. Effect of hypothermia and of cold exposure on adrenal cortical and medullary secretion. Ann. N.Y. Acad. Sci. 80:435-444.

Jansky, L., and J. S. Hart. 1963. Participation of skeletal muscle and kidney during nonshivering thermogenesis in cold acclimated rats. Can. J. Biochem. Physiol. 41:953-964.

Kanter, G. S., R. H. Lubinski, D. Shimandle, and D. A. Dudey. 1963. Regulation of extracellular potassium in hypothermia. Amer. J. Physiol. 205:1285-1289.

Kingsley, G. R., and R. R. Schaffert. 1953. Direct microdetermination of sodium, potassium and calcium in a single biological specimen. Anal. Chem. 25:1738-1741.

Knigge, K. M. 1960. Neuroendocrine mechanisms influencing ACTH and TSH secretion and their role in cold acclimation. Fed. Proc. 19 (Suppl. 5): 45-51.

Knigge, K. M. 1963. Thyroid function and plasma binding during cold exposure of the hamster. Fed. Proc. 22:755-760.

Kunin, A. S. 1963. Skeletal metabolism and electrolyte homeostasis, p. 360-371. In J. H. Bland, Clinical metabolism of body water and electrolytes. W. B. Saunders, Philadelphia, Pa.

Lamberg, B. A., and P. Torsti. 1964. The dependence of calcium excretion on adrenal steroids. Studies with chlorothiazide in patients with impaired adrenal function. Acta. Med. Scand. 175 (Suppl. 412):193-203.

Langdon, L., and D. P. E. Kingsley. 1964. Changes in serum and urinary potassium levels during profound hypothermia in man. J. Clin. Path. 17:257-259.

Laragh, J. H., and H. C. Stoerk. 1957. A study of the mechanism of secretion of the sodium-retaining hormone (Aldosterone). J. Clin. Invest. 36:383-392.

Lee, D. H. K. 1950. Studies of heat regulation in the sheep, with special reference to the Merino. Australian J. Agr. Res. 1:200-216.

Leonard, P. J. 1963. Aldosterone and water and sodium distribution in normal and adrenalectomized rats. J. Endocrinol. 26:525-530.

Magoun, H. W., F. Harrison, J. R. Brobeck, and S. W. Ranson. 1938. Activation of heat loss mechanisms by local heating of the brain. J. Neurophysiol. 1:101-114.

Miller, A. T., Jr. 1955. Comparison of some commonly-used indices of adrenal cortical function. J. Appl. Physiol. 7:660-662.

Moussa, S. L., and A. Boba. 1960. Exogenous plasma magnesium increases during hypothermia in dogs. Amer. J. Physiol. 199:1090-1092.

Munday, K. A., and G. F. Blane. 1960. Changes in electrolytes and 17-oxosteroids in the rat subjected to cold environment. J. Endocrinol. 20:266-275.

Munday, K. A., and G. F. Blane. 1961. Cold stress of the mammal, bird and reptile. Comp. Biochem. Physiol. 2:8-21.

Munday, K. A., G. F. Blane, E. F. Chin, and E. S. Machell. 1958. Plasma electrolyte change in hypothermia. Thorax 13:334-342.

Neubeiser, R. E., W. S. Platner, and J. L. Shields. 1961. Magnesium in blood and tissues during cold acclimation. J. Appl. Physiol. 16: 247-249.

Nicholls, D., and R. J. Rossiter. 1956. Phosphorus metabolism of the adrenal gland of the rat acclimatization to cold. Amer. J. Physiol. 187:11-14.

Nowell, N. W., and D. C. White. 1963. Seasonal variation of magnesium and calcium in serum of the hypothermic rat. J. Appl. Physiol. 18: 967-969.

Nowell, N. W., and D. C. White. 1964. Note on plasma magnesium in the cold-acclimated rat subjected to hypothermia. Can. J. Physiol. Pharmacol. 42:679-680.

Petajan, J. H., P. Morrison, and K. Akert. 1962. Localization of central nervous control of temperature regulation in the opossum. J. Exp. Zool. 150:225-231.

Platner, W. S. 1960. Electrolytes in acclimation to cold. Fed. Proc. 19 (Suppl. 5):130-131.

Platner, W. S., and M. J. Hosko, Jr. 1953. Mobility of serum magnesium in hypothermia. Amer. J. Physiol. 174:273-276.

Platner, W. S., J. L. Sheilds, and F. A. Purdy. 1964. Tissue glycogen fractions of the hypothermic rat, hamster, and turtle. Amer. J. Physiol. 207:42-46.

Pogosova, A. V. 1959. Effect of hypothermia on potassium distribution in the organism. Doklady Akal. Nauk. SSSR 128:209-211. (From Chem. Abstr. 54:9033).

Ranson, C. 1937. Hypothalamic regulation of temperature in the monkey. Arch. Neurol. and Psychiatry 38:445-466.

Rapp, J. P. 1964. Effect of an aldosterone antagonist on electrolytes and juxtaglomerular granularity in adrenal regeneration hypertension. Endocrinology 75:326-332.

Reid, R. L. 1962. Studies on the carbohydrate metabolism of sheep. XV. The adrenal response to the climatic stresses of cold, wind, and rain. Australian J. Agr. Res. 13:296-306.

Reid, R. L., and S. C. Mills, 1962. Studies on the carbohydrate metabolism of sheep. XIV. The adrenal response to psychological stress. Australian J. Agr. Res. 13:282-295.

Repin, I. S. 1963. Significance of the preoptic areas of the brain in chemical thermoregulation in the rabbit. Fiziol. Zhur. SSSR 49: 49-54. (From Biol. Abstr. 43:10437).

Ross, D. N. 1956. Principles underlying the application of hypothermia to cardiac surgery. Roy. Soc. Med. (London), Proc., 49:365-368.

Rovner, D. R., D. H. P. Streeten, L. H. Louis, C. T. Stevenson, and J. W. Conn. 1963. Content and uptake of sodium and potassium in bone. Influence of adrenalectomy, aldosterone, deoxycorticosterone and spironolactone. J. Clin. Endocrinol. 23:938-944.

Scholander, P. F. 1957. "The wonderful net." Sci. Amer. 196:96-107.

Schönbaum, E. 1960. Adrenocortical function in rats exposed to low environmental temperatures. Fed. Proc. 19 (Suppl. 5):85-88.

Scott, D., and A. Dobson. 1965. Aldosterone and the metabolism of magnesium and other minerals in sheep. Quart. J. Exp. Physiol. 50:42-56.

Sellers, E. A., and E. Schönbaum. 1963. Catecholamines in acclimation to cold: historical review. Fed. Proc. 22:909-910.

Selye, H. 1950. The "alarming stimuli" or stressor agents, p. 27-51. In H. Selye, The physiology and pathology of exposure to stress. Acta, Inc., Montreal, Que.

Sims, E. A. H., and S. Solomon. 1963. The role of antidiuretic hormone and of aldosterone in control of water and electrolyte balance, p. 65-89. In J. H. Bland, Clinical metabolism of body water and electrolytes. W. B. Saunders, Philadelphia, Pa.

Slater, J. D. H., B. H. Barbour, H. H. Henderson, A. G. T. Casper, and F. C. Bartter. 1963. Influence of the pituitary and the renin-angiotensin system on the secretion of aldosterone, cortisol, and corticosterone. J. Clin. Invest. 42:1504-1520.

Speirs, R. S., and R. K. Meyer. 1949. The effects of stress, adrenal and adrenocorticotrophic hormones on the circulating eosinophils of mice. Endocrinology 45:403-429.

Spurr, G. B., and G. Barlow. 1959. Plasma and erythrocyte Na, K, Cl and water in hypothermic and hyperthermic dogs. Amer. J. Physiol. 197: 648-652.

Steadman, L. T., I. Ariel, and S. L. Warren. 1943. Studies on the effect of hypothermia. IV. The rise of serum magnesium in rabbits during the hypothermic states as shown by the spectrochemical method. Cancer Res. 3:471-474.

Steel, R. G. D., and J. H. Torrie. 1960. Duncan's new multiple range test, p. 107-109. In R. G. D. Steel and J. H. Torrie, Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, N.Y.

Swan, H., I. Zeavin, J. H. Holmes, and V. Montgomery. 1953. Cessation of circulation in general hypothermia. I. Physiologic changes and their control. Ann. Surg. 138:360-376.

Swingle, W. W., and A. J. Swingle. 1965. Effect of adrenal steroids upon plasma volume of intact and adrenalectomized dogs. Proc. Soc. Exp. Biol. Med. 119:452-455.

Veeraraghavan, G. 1963. A study of diurnal temperature patterns in sheep. M.Sc. Thesis, University of Alberta, Edmonton, Alberta.

Weeth, H. J., C. R. Torell, and D. W. Cassard. 1959. Effects of a simulated snowbound stress condition on ewes. J. Animal Sci. 18: 694-700.

White, A., P. Handler, E. L. Smith, and D. Stetten, Jr. 1959. Biochemistry of the endocrine glands, p. 853-973. In A. White, P. Handler, E. L. Smith, and D. Stetten, Principles of biochemistry. 2nd ed. McGraw-Hill Book Co., Inc., New York, N.Y.

Wynn, V. 1954. Electrolyte disturbances associated with failure to metabolise glucose during hypothermia. Lancet 2:575-578.

Yamashita, K., and M. Araki. 1962. Relationship between eosinopenic response to cold application and the adrenal gland in the dog. Acta Med. Nagasak. 6:91-99. (From Biol. Abstr. 43:5431).

APPENDIX I

Construction of Metabolism Cages

The metabolism cages were constructed of Dexion angle steel and 3/8-inch plywood as illustrated in Fig. 1. The cages had 2.5-ft high sides and floor dimensions of 2 ft x 4.5 feet. The cages were made with 2-ft legs to allow clearance for the urine collection funnel and reservoir. The feed box was made of 3/8-inch plywood and could be lifted out easily. The feed box was designed to hold a plastic 2-gal pail for water.

The feces tray and the urine funnel were made from 16-gauge galvanized tin and were designed to slide out for cleaning. The floor above the urine funnel was made from 12-gauge flattened expanded steel with 1/2 x 3/4-inch openings.

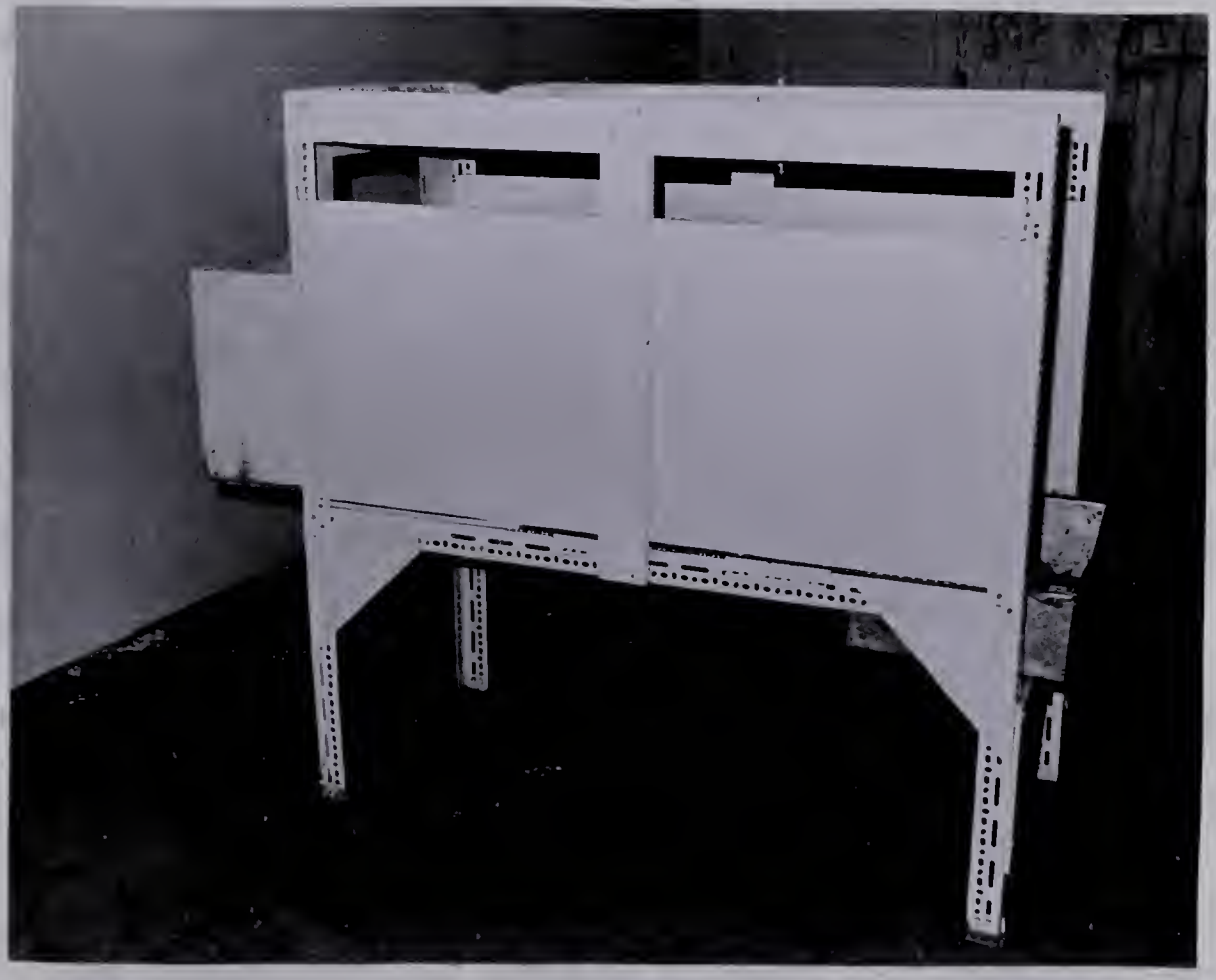


Fig. 1. Side view of a metabolism cage

APPENDIX II

Construction of the Cold Chamber

The chamber was 4 ft wide, 8 ft long, and 7 ft high. This left room enough to manoeuvre around inside it if the metabolism cage was placed against one wall.

The framework was made of 2 x 4-inch lumber. The inner surfaces of the walls and ceiling were sheathed with 3/8-inch plywood. The walls and ceiling were insulated with 3-inch fiberglass batting. The outer surface of the walls and ceiling were covered with polyethylene which served as a vapour seal for the insulation. The chamber was then sheathed over with 1/4-inch plywood. The floor was made with a bottom layer of 3/8-inch plywood, a layer of polyethylene vapour seal, a layer of vermiculite insulation, and a floor of 3/4-inch plywood. The door for the front of the chamber was insulated with fiberglass and made of two 3/8-inch plywood layers. A smaller door, 2.5 x 4 ft, was cut out of the main door. The small door was easy to remove and was held in place by bolts and wing nuts.

The chamber was designed to be air-tight except for two 2-inch ventilation ports. Air exchange was effected by a 100-cfm capacity electric blower. A stopper with a section of 1-inch plastic pipe inserted through it was placed in the exhaust port to restrict the exit air flow. When controlled in this manner, the rate of air exchange was approximately 140 liters/minute.

The temperature was lowered by means of a water-cooled 1/3 horse-power compressor[1] mounted outside the chamber (Fig. 1). The evaporator fan-coil unit was hung from the ceiling of the chamber, near the back (Fig. 2).

[1]Copeland Refrig. Corp., Sidney, Ohio, USA.

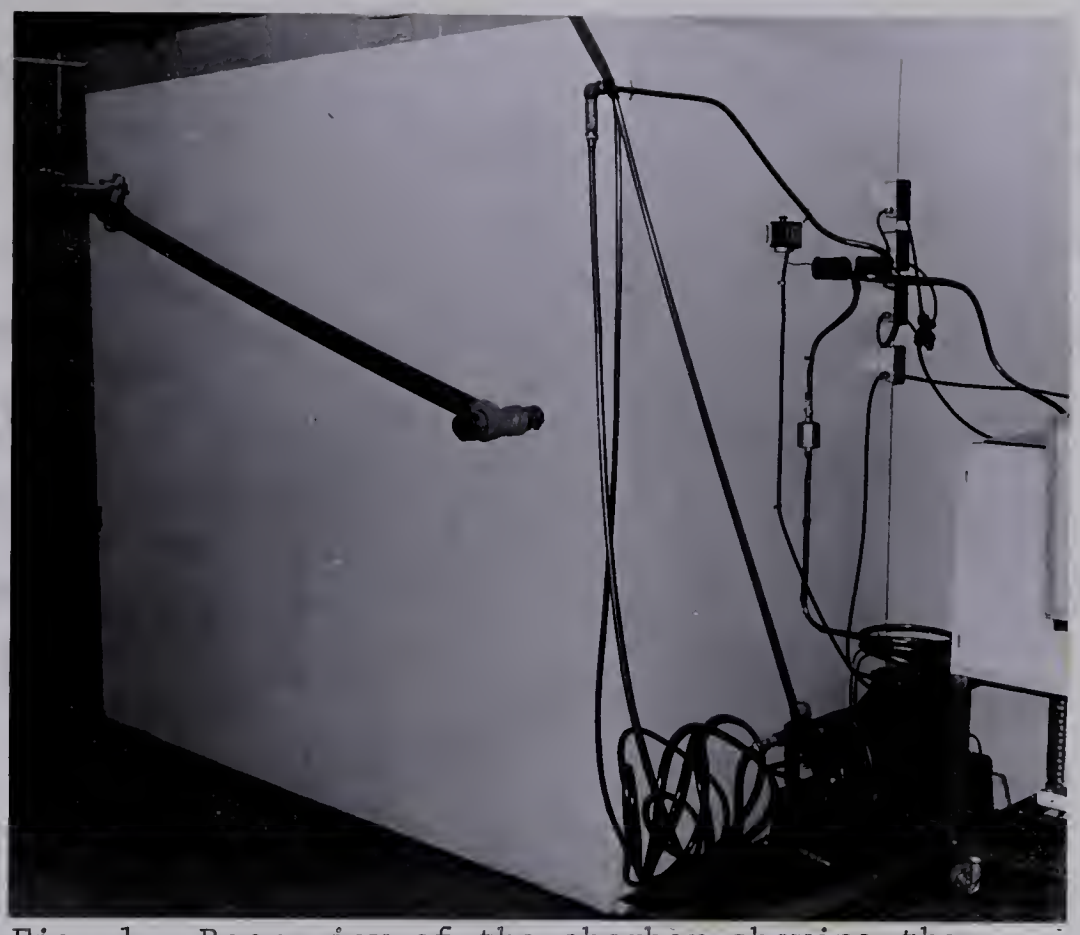


Fig. 1. Rear view of the chamber showing the compressor unit

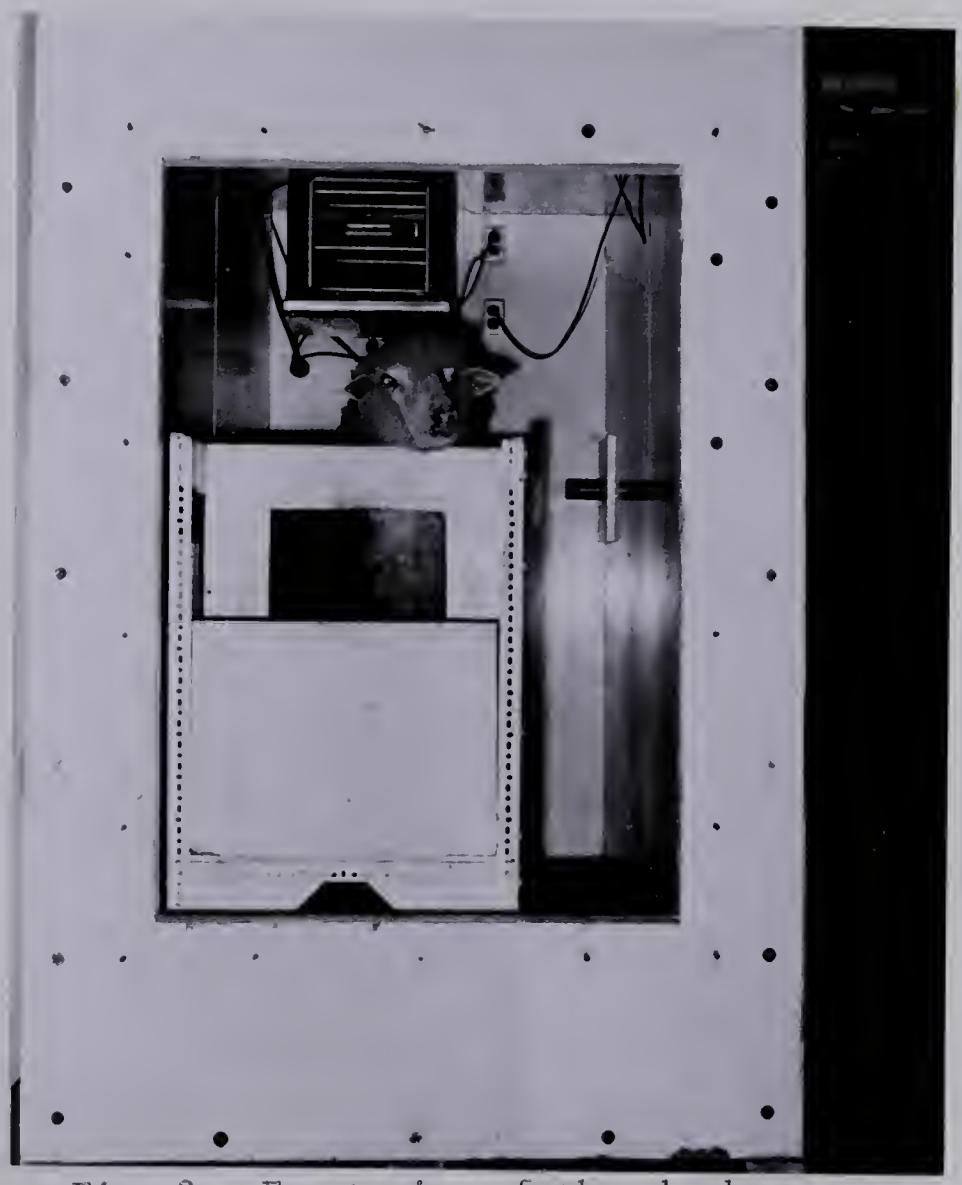


Fig. 2. Front view of the chamber showing the evaporator fan-coil unit

With a fully grown wether in the chamber, the refrigeration unit was able to maintain a temperature of approximately 0 C. The relative humidity of the chamber air was 76% during the cold period and 78% during the rewarm period (Appendix III, Table 5). This was significantly ($P < 0.01$) higher than the 68% relative humidity observed in the chamber during the control period (Appendix III, Table 1).

The average relative humidity at each sampling time is reported in Table 1.

Table 1. Mean relative humidity (%) in chamber during four trials

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	67.3	70.5	71.0	70.5	73.3	70.3	68.0	66.3	65.8	66.5	63.5	68.8	64.0	69.0
SD	5.1	4.4	5.0	3.0	3.0	4.8	3.4	2.2	1.5	1.0	2.9	5.9	3.8	5.9
Cold	67.5	64.5	73.3	72.3	75.3	75.8	78.8	79.8	73.3	78.8	78.3	81.3	82.8	79.0
SD	5.1	2.9	9.5	7.1	12.2	5.7	9.7	6.9	9.8	3.1	4.1	1.7	5.1	4.3
Rewarm	81.8	95.0	90.0	83.5	86.0	83.0	77.3	79.3	74.8	67.5	67.3	66.3	69.5	70.3
SD	7.0	2.7	5.6	6.4	4.9	8.7	2.6	2.2	7.8	2.7	2.2	3.9	2.9	2.4

| | | |

APPENDIX III

Table 1. Mean squares — The effect of ambient temperature on chamber relative humidity

Source of variance	df	Relative humidity
Total	167	
Sheep (S)	3	6.1
Treatment (T)	2	1500**
Sample (Sa)	13	91**
S x T	6	100**
S x Sa	39	28
T x Sa	26	190**
Residual	78	25

**$P < 0.01$.

Table 2. Mean squares — The effect of ambient temperature on jugular vein and rectal temperatures

Source of variance	df	JVT[a]	df	RT[b]
Total	83		167	
Sheep (S)	1	2.23**	3	0.613**
Treatment (T)	2	0.159*	2	0.470**
Sample (Sa)	13	0.027	13	0.025
S x T	2	0.482**	6	0.454**
S x Sa	13	0.022	39	0.035
T x Sa	26	0.017	26	0.043
Residual	26	0.047	78	0.031

[a]Jugular vein temperature.

[b]Rectal temperature.

*$P < 0.05$.

**$P < 0.01$.

Table 3. Mean squares — The effect of ambient temperature on packed cell volume, eosinophil count, and plasma glucose

Source of variance	df	PCV[a]	EC[b]	Glucose
Total	167			
Sheep (S)	3	24.7**	685,000**	66.8**
Treatment (T)	2	101.0**	41,400**	161**
Sample (Sa)	13	6.60**	1,960	15.9
S x T	6	22.6**	9,350	48.9**
S x Sa	39	2.63	1,200	12.6
T x Sa	26	7.17**	14,800**	14.4
Residual	78	2.07	4,560	16.1

[a]Packed cell volume.
[b]Eosinophil count.
**P < 0.01.

Table 4. Mean squares — The effect of ambient temperature on plasma sodium, potassium, calcium, and magnesium

Source of variance	df	Sodium	Potassium	Calcium	Magnesium
Total	167				
Sheep (S)	3	329.8**	4.40**	2.59**	0.216**
Treatment (T)	2	626.3**	2.68**	0.111**	0.252**
Sample (Sa)	13	45.11**	0.172	0.064**	0.020**
S x T	6	293.6**	0.689**	0.532**	0.065**
S x Sa	39	18.57**	0.083	0.022	0.006*
T x Sa	26	27.84**	0.111	0.034**	0.007*
Residual	78	7.411	0.094	0.015	0.004

*P < 0.05.
**P < 0.01.

Table 5. Period means for the ten parameters determined during the experiment

Parameter	Control	Cold	Rewarm
Packed cell volume	34.0	34.4	31.9
Eosinophils (per mm^3)	203	151	164
Plasma sodium (meq/l)	150.5	155.2	156.9
Plasma potassium (meq/l)	4.76	4.95	4.52
Plasma calcium (meq/l)	5.01	5.02	4.94
Plasma magnesium (meq/l)	1.81	1.77	1.68
Plasma glucose (mg/100 ml)	64.6	67.1	63.8
Rectal temperature (C)	39.0	38.8	38.9
Jugular vein temperature (C)	38.5	38.4	38.5
Relative humidity (%)	68	76	78

Table 6. Mean[a] packed cell volumes (% of whole-blood volume)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	35.9	36.3	34.8	34.5	34.6	34.0	35.2	34.7	33.3	33.2	32.7	33.2	32.2	32.2
SE	1.4	1.6	1.4	1.4	1.6	1.2	1.7	1.5	1.5	0.3	0.3	0.7	0.6	0.9
Cold	32.8	32.5	33.5	34.8	34.9	33.6	34.6	35.0	33.8	35.0	36.0	34.8	34.6	35.1
SE	0.4	0.8	0.4	0.8	1.1	0.8	1.0	1.0	1.0	1.1	0.5	0.8	0.8	0.8
Rewarm	35.5	33.6	33.1	33.0	32.5	32.5	31.8	31.5	31.2	31.6	30.6	29.9	29.9	29.8
SE	0.4	0.6	0.1	0.7	0.9	1.0	1.1	1.2	0.6	0.8	0.5	0.5	0.8	0.8

[a] Four sheep.

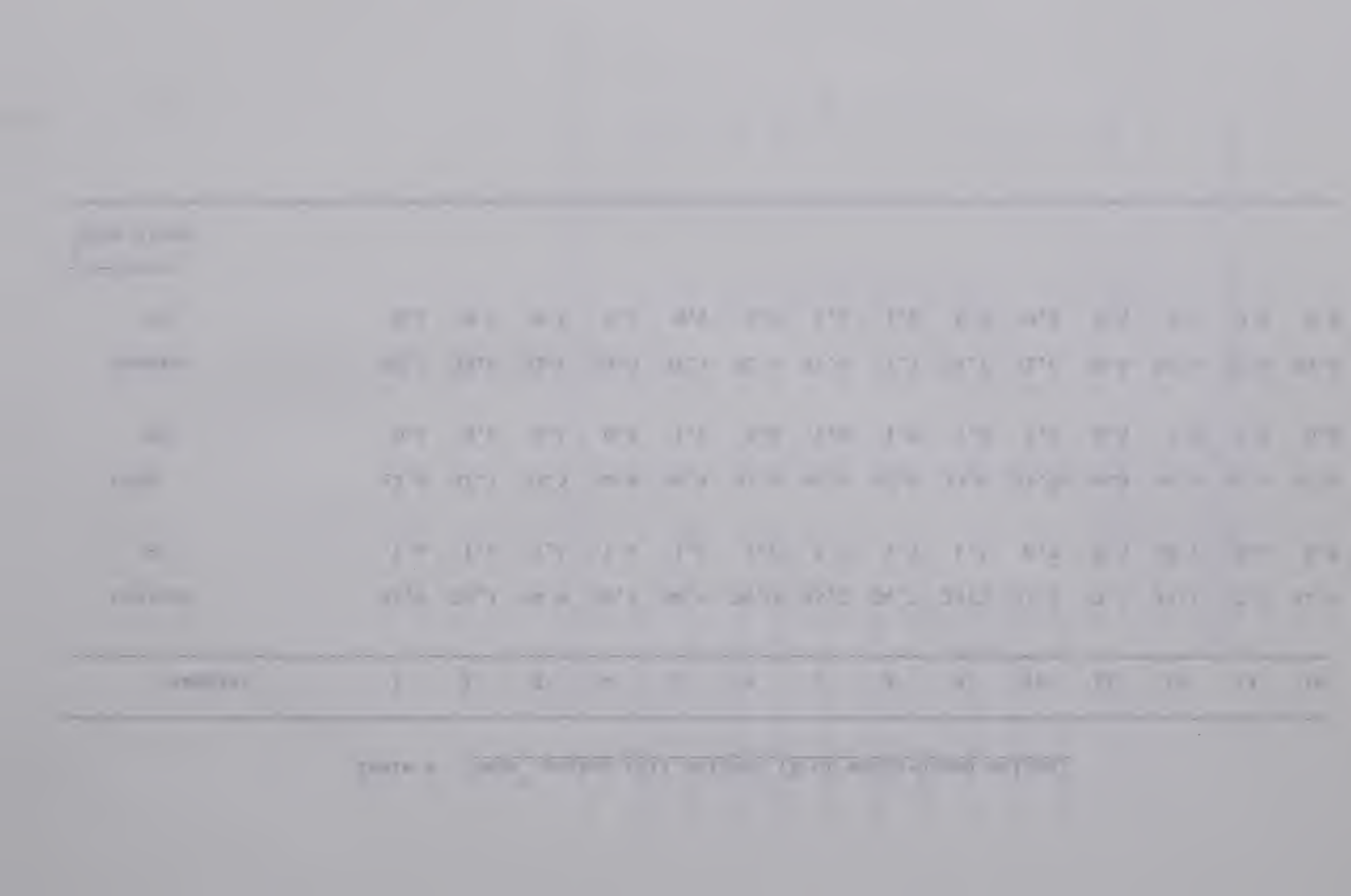


Table 7. Mean[a] eosinophil counts (cells/mm^3)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	180	192	174	218	189	174	182	182	192	203	224	250	254	229
SE	47	74	60	84	73	68	63	69	61	58	93	104	103	96
Cold	220	244	253	216	184	160	159	139	100	97	88	75	88	91
SE	77	100	104	87	67	67	63	54	26	28	19	20	30	20
Rewarm	104	102	93	134	123	120	149	148	178	237	234	245	196	237
SE	13	43	42	63	65	48	66	65	81	96	102	93	71	96

[a] Four sheep.

| | | |

Table 8. Mean[a] plasma sodium concentrations (meq/l)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	151.6	152.4	152.5	153.0	154.2	152.8	151.8	152.5	150.8	147.0	147.2	145.9	148.2	146.5
SE	2.1	2.6	3.9	4.0	3.1	3.6	3.0	4.1	4.1	3.6	3.8	2.0	1.1	1.2
Cold	151.2	151.8	155.4	155.7	155.8	154.9	155.5	154.9	157.1	155.2	156.4	156.0	156.2	156.1
SE	2.4	1.3	4.2	4.4	4.8	3.7	3.3	4.0	3.7	1.4	1.7	1.2	0.8	1.2
Rewarm	159.8	161.6	162.8	161.2	160.3	157.7	158.3	155.4	155.4	153.2	154.1	153.4	151.8	152.0
SE	3.6	4.2	4.3	2.4	3.1	2.8	2.9	1.7	2.0	1.7	0.4	0.8	0.9	0.3

[a] Four sheep.

Table 9. Mean[a] plasma potassium concentrations (meq/l)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	5.02	4.77	4.82	4.69	4.89	4.76	5.14	5.00	4.69	4.58	4.51	4.61	4.70	4.48
SE	0.15	0.17	0.31	0.20	0.38	0.34	0.38	0.20	0.38	0.13	0.18	0.07	0.17	0.20
Cold	4.91	4.92	5.47	5.04	5.13	4.84	5.07	4.78	4.92	4.85	5.06	5.03	4.58	4.73
SE	0.17	0.31	0.43	0.19	0.28	0.10	0.24	0.34	0.20	0.17	0.30	0.25	0.28	0.12
Rewarm	4.66	4.65	4.55	4.55	4.63	4.40	4.59	4.38	4.35	4.43	4.31	4.47	4.56	4.69
SE	0.32	0.24	0.10	0.10	0.16	0.16	0.31	0.24	0.18	0.14	0.13	0.18	0.20	0.17

[a]Four sheep.

Table 10. Mean[a] plasma calcium concentrations (meq/l)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	4.99	5.05	5.08	5.10	5.12	4.91	5.06	5.09	4.95	5.06	4.98	4.86	5.00	4.93
SE	0.15	0.19	0.25	0.25	0.24	0.34	0.28	0.24	0.23	0.22	0.21	0.20	0.24	0.18
Cold	4.90	4.99	5.05	5.08	5.13	5.02	5.01	5.02	5.00	4.97	4.97	5.08	5.01	5.00
SE	0.11	0.13	0.16	0.14	0.12	0.16	0.14	0.16	0.13	0.12	0.09	0.09	0.10	0.11
Rewarm	5.11	5.16	5.14	5.00	5.07	5.01	4.99	4.94	4.91	4.81	4.74	4.76	4.73	4.77
SE	0.09	0.07	0.10	0.11	0.05	0.06	0.06	0.03	0.08	0.10	0.11	0.12	0.12	0.12

[a] Four sheep.

Table 11. Mean[a] plasma magnesium concentrations (meq/l)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	1.79	1.80	1.85	1.88	1.86	1.86	1.87	1.89	1.80	1.79	1.77	1.72	1.72	1.70
SE	0.03	0.04	0.06	0.06	0.09	0.06	0.08	0.04	0.05	0.07	0.06	0.08	0.06	0.06
Cold	1.77	1.76	1.80	1.79	1.77	1.80	1.82	1.76	1.77	1.78	1.78	1.79	1.70	1.73
SE	0.05	0.05	0.05	0.05	0.05	0.03	0.05	0.06	0.06	0.05	0.09	0.07	0.06	0.05
Rewarm	1.80	1.75	1.74	1.72	1.68	1.71	1.68	1.68	1.59	1.61	1.63	1.64	1.65	1.63
SE	0.08	0.06	0.03	0.03	0.03	0.02	0.02	0.05	0.06	0.07	0.08	0.07	0.07	0.05

[a]Four sheep.

Table 12. Mean[a] plasma glucose concentrations (mg/100 ml)

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	65.2	68.3	66.5	66.3	64.4	65.0	64.8	65.3	66.9	64.2	62.1	61.7	62.1	62.0
SE	0.9	1.9	0.5	1.2	0.7	1.3	0.6	1.3	1.0	1.3	1.1	3.2	1.1	1.0
Cold	62.9	64.6	67.2	67.6	67.2	68.8	70.0	69.7	69.7	66.6	65.2	66.3	66.9	66.7
SE	1.5	1.1	1.9	2.1	1.5	1.8	2.1	2.1	2.1	2.1	1.1	2.6	1.5	0.5
Rewarm	65.3	67.6	60.1	65.0	63.5	63.6	63.7	64.1	63.2	63.7	63.5	61.9	64.3	64.2
SE	0.8	2.4	8.4	2.1	3.7	1.2	2.6	1.1	1.2	0.7	0.8	1.0	1.1	2.3

[a]Four sheep.

APPENDIX IV

Table 1. Mean feed consumption, water consumption, and urine excretion

	Control		Cold		Rewarm	
Pellets[a]	2,229	472	1,925	362	2,320	635
Water[b]	3,831	1,110	1,638	985	5,749	2,210
Urine[b]	1,102	32	894	195	1,204	240

[a] Grams per day ± 1 SD.

[b] Milliliters per day ± 1 SD.

Table 2. Mean squares — The effect of ambient temperature on feed consumption, water consumption, and urine excretion

Source of variance	df	Pellets	Water	Urine
Total	8			
Sheep	2	674,138*	2,727,861	13,884
Treatment	2	128,523	12,696,066*	75,230
Error	4	85,043	975,395	13,264

* $P < 0.05$.

APPENDIX V

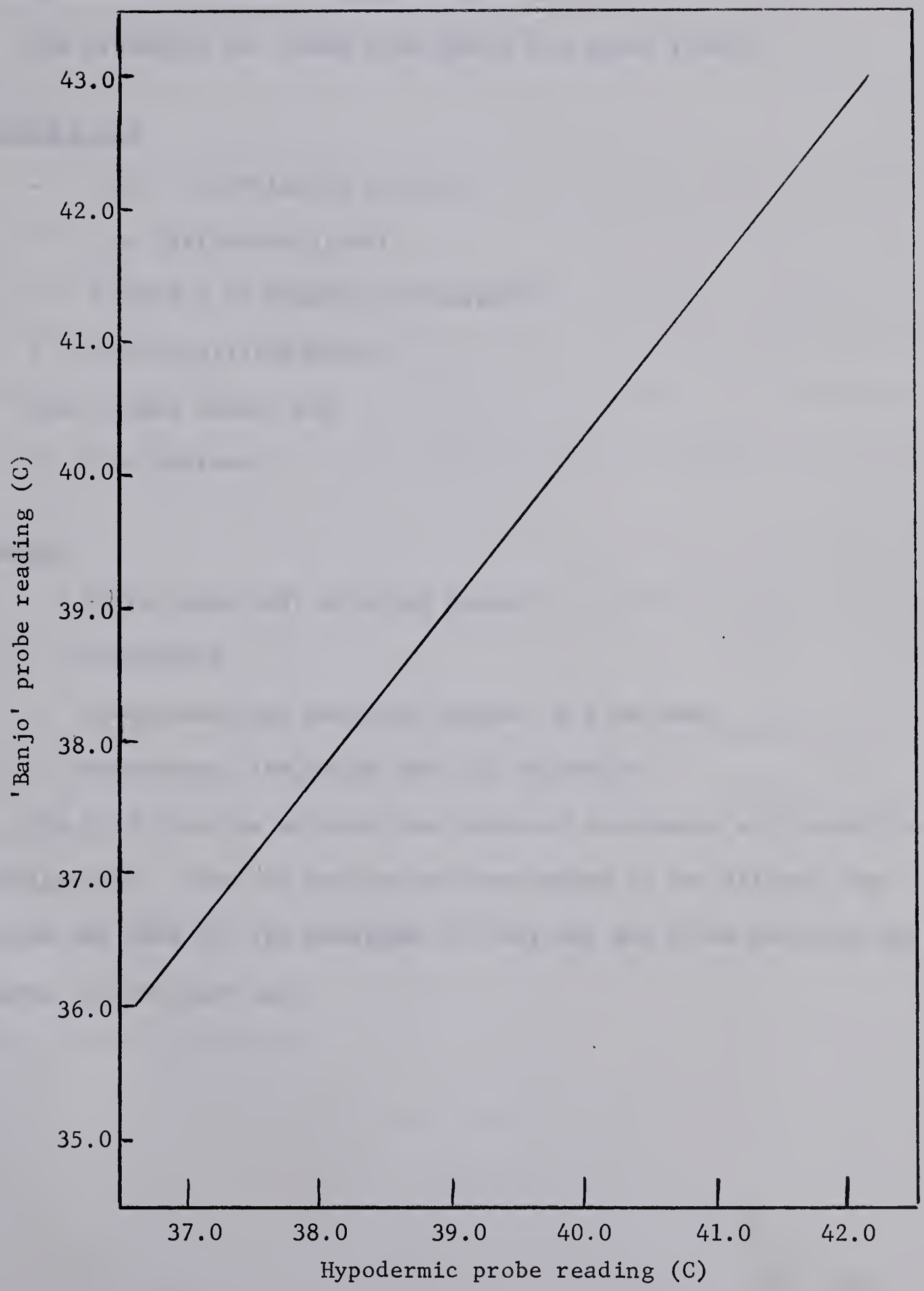
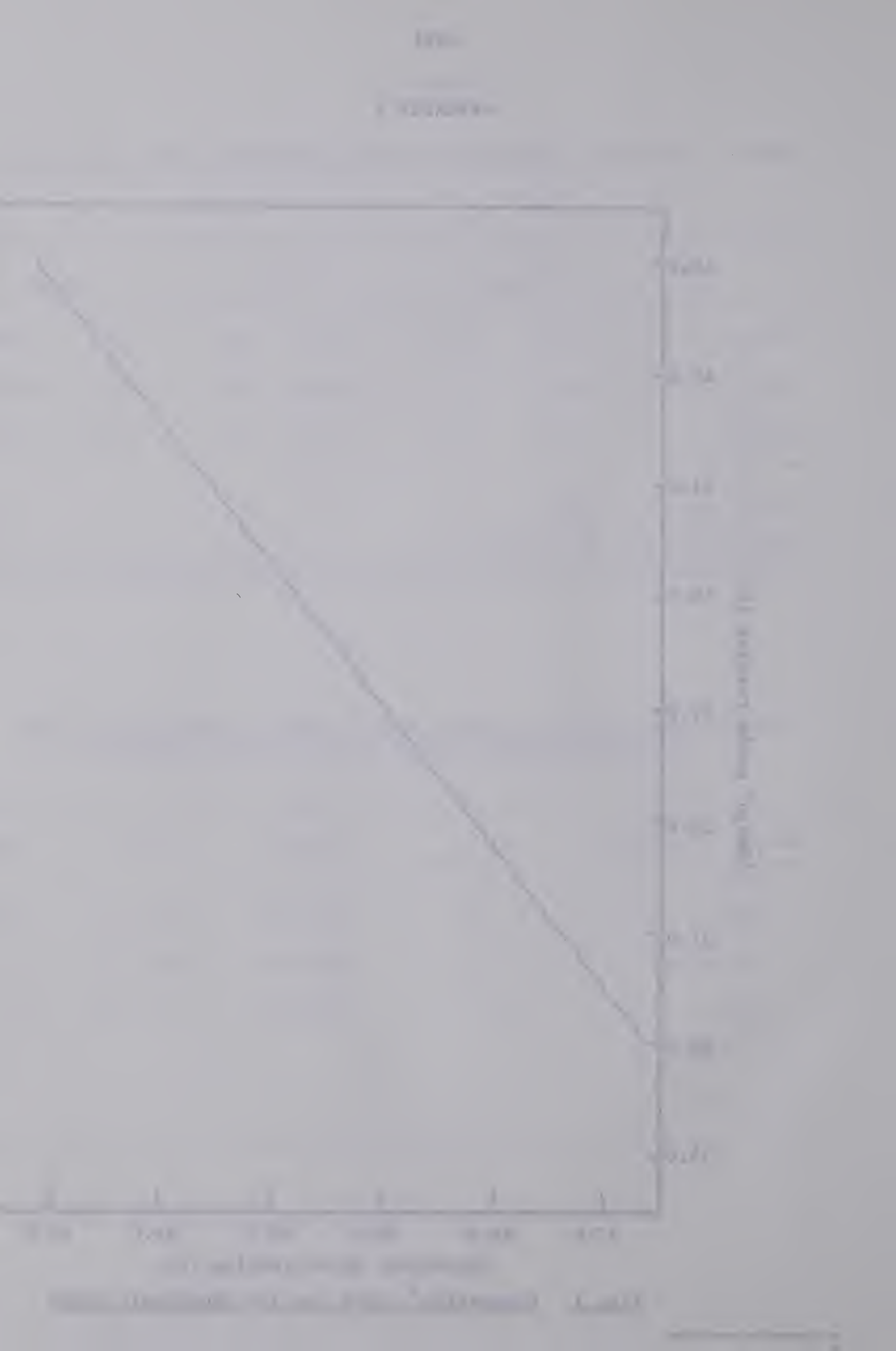


Fig. 1. Conversion[a] curve for the hypodermic probe

[a] Corrected against the 'Banjo' probe which was previously verified by a mercury thermometer.



APPENDIX VI

Eosinophil Counting Diluent and Equipment

The procedure was taken from Speirs and Meyer (1949).

Diluting fluid

- 10 cc 0.1% Phloxive solution
- 1 cc diethylene glycol
- 4 drops 0.5% Dynaklen (detergent)
- 30 cc distilled water

Just before using, add

- 7 cc acetone

Equipment

- White blood cell diluting pipets
- Aspirators
- Fuchs-Rosenthal counting chamber, 0.2 mm deep
- Microscope, 10X ocular and 10X objective

The 0.1% Phloxine solution was prepared in advance and stored in a refrigerator. Once the acetone had been added to the diluent, the solution was used for the remainder of that day and a new solution was prepared for the next day.

APPENDIX VI

Flame Photometry Reagents and Formulas

The procedure was taken from Kingsley and Schaffert (1953).

Solutions

a) Mixed-Stock Standard (150 meq/1 of sodium, 5 meq/1 of calcium, and 5 meq/1 of potassium): first, 0.2496 g of calcium carbonate were dissolved in 10 ml of concentrated hydrochloric acid in a 1-liter volumetric flask. This was diluted to about 500 ml with deionized water. To this, 8.7690 g of dry sodium chloride and 0.3728 g of dry potassium chloride were added. These were dissolved and the solution was diluted to 1 liter at room temperature.

b) Sterox Solution (0.02%): 0.2 ml of Sterox was dissolved in 1 liter of deionized water.

c) Working standards were prepared by diluting appropriate volumes of the stock standard with the 0.02% solution of Sterox in deionized water.

All solutions were stored in polyethylene bottles.

Sodium formula calculations

$$Rs \times 2 - (Rs - Ru)4 = \text{meq/1 of Na}$$

$$4\,Ru - 2\,Rs = \text{meq/1 of Na}$$

$$4\,Ru - 150 = \text{meq/1 of Na , where}$$

Ru = unknown reading and Rs = reading of the 150 meq/1 sodium standard.

This is an empirical formula derived from the slope of the standard curve obtained when the 100 meq/l sodium standard reads 62.5% T.

Potassium formula calculations

Since the standard curve for the flame photometer was a straight line beginning at the origin for both potassium and calcium, the formula used was:

$$\frac{Ru}{Rs} = \frac{Mu}{Ms}$$

$$\frac{Ru}{50} = (\text{meq/l of K or Ca})(1/5)$$

$$\frac{Ru}{10} = \text{meq/l of K or Ca , where}$$

Ru = unknown reading, Rs = standard reading, Mu = meq/l in the unknown, and Ms = meq/l in the standard.

APPENDIX VI

Titration Reagents for Calcium and Magnesium

The procedure was taken from Carr and Frank (1956).

The reagents were prepared with deionized water. The standards and reagents were stored in polyethylene bottles.

a) Stock Standard for Calcium (100 meq/1): calcium carbonate was dried to a constant weight at 110 C and 2.502 g of it were dissolved in 5 to 10 ml of concentrated hydrochloric acid. The solution was then diluted to 500 milliliters.

b) Stock Standard for Magnesium (40 meq/1): magnesium oxide was heated in a platinum crucible until a constant weight was obtained and 0.4032 g of it were dissolved in 5 to 10 ml of concentrated hydrochloric acid. The solution was then diluted to 500 milliliters.

c) Mixed Working Standard (calcium, 5 meq/1; magnesium, 2 meq/1): 5 ml of each of the stock solutions were mixed and diluted to 100 milliliters.

d) Saturated Solution of Ammonium Oxalate: a 500-ml saturated ammonium oxalate solution was prepared by heating the mixture until the solute dissolved and then allowing it to cool to room temperature.

e) Solution of Ammonium Hydroxide: 10 ml of concentrated ammonium hydroxide (28%) was diluted to 500 milliliters.

(2.319 M)

f) Solution of Perchloric Acid: 20 ml of reagent-grade perchloric acid (70%) were diluted to 100 milliliters.

Translation Programs for Decision Tree Induction

The procedures are taken from Quinlan (1986).

The routines have provision for handling noise. The principle and treatment used rather is polynomial basis.

a) Check Consistency for Training (CCT mode): Training continues until defined is a consistent subset or the j and E(X) j is less than or equal to j as the percentage misclassified by the majority class. The minimum may be defined by the utilities.

b) Trees Pruning for Reduction (Pr mode): Reduction trees is limited to a j critical error rate until a minimal subset has been found. (CCT) j or is more accurate in j to or of consistent information rule. The selection may then utilized by the utilities.

c) Rule Making Started (Pattern) mode): Reducing j may j or of each of the rules solutions over terms are utilized in the utilities (item).

d) Checking Solution if (Decision Option): j Boolean information complete existing minimum may propose by testing the classes over the values discovered and then utility of the last to form complete.

e) Checking of Decision Information: to be of consistent sensitive (Decision (CDI) may utilized by the utilities.

f) Creation of Decision Rule: to of of consistent sensitive hold (CDI) rule rules by the utilities.

(0.655 M)

g) Solution of Ethanolamine: reagent-grade ethanolamine was redistilled under vacuum in a fume cabinet. The redistilled ethanolamine was stored in a dark cupboard. Ten ml of the redistilled ethanolamine was diluted to 250 milliliters. The ethanolamine solution was freshly prepared every day.

h) Magnesium - EDTA Complex: 2 g of magnesium chloride was dissolved in 25 ml of water and 7.5 g of disodium ethylenediamine tetraacetate (EDTA) were dissolved in 75 ml of water. These solutions were mixed and when precipitation was complete the mixture was filtered. The complex was washed several times with small quantities of cold distilled water and then dried at room temperature.

i) Ethanolamine - Magnesium - EDTA Complex: about 10 mg of the magnesium - EDTA complex were dissolved in 100 ml of the solution of ethanolamine and were freshly prepared every day.

(0.012 M)

j) Stock Solution of EDTA: this was prepared by dissolving 4.5 g of reagent-grade EDTA in water and diluting to 1 liter.

k) Working Solution of EDTA for Titration of Calcium: 50 ml of the stock solution of EDTA were diluted to 500 milliliters.

1) Working Solution of EDTA for Titration of Magnesium: 25 ml of the stock solution of EDTA were diluted to 500 milliliters.

m) Solution of Eriochrome Black T (EBT): every 2 weeks a new solution of EBT was prepared by dissolving 0.1 g of EBT in 25 ml of methanol and adding 1 ml of concentrated ammonium hydroxide.

APPENDIX VII

A Comparison of the Two Methods Used for Calcium Determination

The t test was used to compare the results obtained by the two methods used for calcium determination. For two sets of samples the differences between the methods were nonsignificant. These samples were analyzed by the two methods over a 2-week period. However, the other two sets of samples were analyzed by the volumetric method several months previous to the flame photometric analysis. The volumetric results in this case averaged about 0.5 meq/l higher. These samples had been thawed and refrozen twice in the interval between the two analyses and a crystalline substance was formed around the top of the tubes. When this was dissolved and analyzed it was found to be high in calcium.

The values for calcium which are reported throughout this project are those obtained by the flame photometric method.

APPENDIX VII

A Comparison of the Two Methods Used for Calcium Determination

The t test was used to compare the results obtained by the two methods used for calcium determination. For two lots of sampled the differences between the methods were insignificant. These samples were analyzed by the two methods over a 7-week period. However, the other two sets of samples were analyzed by the autoanalyzer method several months previous to the flame photometric analysis. The polarographic results in this case averaged about 7.5 meq/l higher. These samples had been thawed and refrozen prior to the initial determination by the two analyses and a crystalline precipitate was formed around the top of the tubes. When this was dissolved and analyzed it was found to be high in calcium.

The values for calcium which are reported throughout this investigation are those obtained by the flame photometric method.